Volume 68
Number 8 / Numéro 8
August / Août
2011

An NRC Research Press Journal
Un journal de NRC Research Press

www.nrcresearchpress.com

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

NRC
Research Press

ISSN 0706-652X / CJFSDX 68(8) 1307–1510 (2011)

The *Canadian Journal of Fisheries and Aquatic Sciences*, previously published as *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, and *Journal of the Fisheries Research Board of Canada* 1938–1979, is issued monthly to form annual volumes of 12 numbers plus supplements.

Editorial policy. The Journal publishes original research articles, perspectives (syntheses, critiques, reevaluations), discussions (comment and reply), and rapid communications. Manuscripts may concern cells, organisms, populations, communities, ecosystems, or processes that affect aquatic systems. They may cover a range of disciplines including biology and ecology of marine and freshwater organisms, limnology, oceanography, physiology, toxicology, genetics, economics, disease, and management. Preference will be given to manuscripts that emphasize understanding of observed phenomena and interpretation of experimental results. The Journal encourages papers that lead from clearly stated purpose or rationale and from testable hypotheses, concepts, or questions to identifiable conclusions or syntheses. Such papers may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. The Journal discourages papers that are essentially descriptive, except in emerging disciplines; have only site-specific or local applications (a certain year, place, taxon, chemical compound, etc.); only confirm previously established principles; or apply standard techniques without breaking new methodological ground.

Manuscripts for publication should be submitted to the Editorial Office via NRC Research Press' online submission and peer review system (**ScholarOne**) at mc.manuscriptcentral.com/cjfas-pubs.

Submissions in either English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editors.

Correspondence concerning accepted manuscripts and corrected proofs should be sent to NRC Research Press, Ottawa, ON K1A 0R6, Canada (fax: 613-952-7656; e-mail: pubs@nrcresearchpress.com).

Instructions to Authors are published at nrcresearchpress.com/page/cjfas/authors. The **Publishing Policy** of NRC Research Press is available at nrcresearchpress.com/page/about/policies/pub.

The ***Canadian Journal of Fisheries and Aquatic Sciences*, ISSN 0706-652X**, is published **Monthly, 12 times per year**, by **NRC Research Press, c/o USACAN Media Dist. Srv. Corp.** at 26 Power Dam Way, Suite S1-S3, Plattsburgh, NY 12901, USA. Periodicals postage paid at Plattsburgh, NY. POSTMASTER: Send address changes to ***Canadian Journal of Fisheries and Aquatic Sciences***, c/o NRC Research Press, P.O. Box 2888, Plattsburgh, NY 12901, USA.

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, et *Journal of the Fisheries Research Board of Canada* 1938–1979. Le Journal paraît tous les mois, formant ainsi un volume de 12 numéros, et offre en plus jusqu'à deux suppléments par année.

Politique éditoriale. On publie, dans le Journal, des articles rendant compte de recherche originale, des perspectives (synthèses, critiques et réévaluations), des discussions (commentaires et réponses) et des communications rapides. Les documents peuvent porter sur les cellules, organismes, populations, communautés, écosystèmes ou processus qui affectent les systèmes aquatiques. Ils peuvent relever de divers domaines y compris la biologie et l'écologie des organismes marins et d'eau douce, la limnologie, l'océanographie, la physiologie, la toxicologie, la génétique, l'économie, la pathologie et la gestion. On accorde la préséance aux articles qui contribuent à la compréhension de phénomènes observés et à l'interprétation de résultats expérimentaux. On invite les auteurs à soumettre des articles ayant des objectifs précis ou relevant d'hypothèses, de concepts ou de questions vérifiables et se terminant par de solides conclusions ou d'utiles synthèses. Ces articles enrichissent, changent, mettent en question ou réorientent les connaissances acquises sur divers aspects des sciences aquatiques. On décourage toutefois la soumission d'articles simplement descriptifs (à moins qu'il ne s'agisse de nouvelles disciplines), d'application très limitée (applicables à une seule année, une seule région ou un seul taxon, portant sur un unique composé chimique, etc.); d'articles qui ne font que reprendre les principes énoncés dans des articles précédents; et d'articles rendant compte de l'application de normes sans gain de méthodologie.

Les auteurs sont invités à soumettre leur **manuscrit** au Bureau de la rédaction par le truchement du Système de soumission et d'examen en direct des articles (**ScholarOne**) de NRC Research Press au mc.manuscriptcentral.com/cjfas-pubs.

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à.-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement des rédacteurs en chef.

La correspondance ayant trait aux manuscrits acceptés et les épreuves corrigés doivent être envoyées à NRC Research Press, Ottawa, ON K1A 0R6, Canada (télécopieur : 613-952-7656; courriel : pubs@nrcresearchpress.com).

Les **Recommandations aux auteurs** sont publiées au nrcresearchpress.com/page/cjfas/authors. La **Politique éditoriale** est disponible auprès de NRC Research Press au nrcresearchpress.com/page/about/policies/pub.

This Journal is indexed or abstracted in / Cette revue est signalée ou résumée dans
Aqualine; Aquatic Sciences and Fisheries Abstracts; BIOBASE; Biological and Agricultural Index; BIOSIS; CAB Abstracts; Canadian Fisheries Abstracts; Chemical Abstracts; Current Awareness in Biological Sciences; Current Abstracts; Current Contents; General Science Index; GeoRef; Oceanic Abstracts; PASCAL; Pollution Abstracts; Referativnyi Zhurnal; Science Citation Index; Water Resources Abstracts

Volume 68, Number 8, August 2011

Canadian Journal of Fisheries and Aquatic Sciences

Published by the NRC Research Press

Volume 68, numéro 8, août 2011

Journal canadien des sciences halieutiques et aquatiques

Publié par NRC Research Press

Editors / Rédacteurs en chef Don Jackson and Rolf Vinebrooke

Assistant to the Editors / Adjointe au rédacteur en chef **Holly Foster**

Associate Editors / Comité de rédaction

M.J. Bradford, *Simon Fraser University*
D. Brickman, *Fisheries and Oceans Canada, Dartmouth*
S.E. Campana, *Fisheries and Oceans Canada, Dartmouth*
Y. Chen, *University of Maine*
I. Fleming, *Memorial University of Newfoundland*
D. Fraser, *Concordia University*
B.M. Gillanders, *University of Adelaide*
J.W.A. Grant, *Concordia University*
M.M. Hansen, *Aarhus University*
R. Hilborn, *University of Washington*
J.M. Jech, *National Marine Fisheries Service, NOAA, Woods Hole, Mass.*
B. Jonsson, *Norwegian Institute for Nature Research*
J. Karlsson, *Umeå University*
K.A. Kidd, *University of New Brunswick*
C.E. Kraft, *Cornell University*
D.L. MacLatchy, *Wilfrid Laurier University*
P. Magnan, *Université du Québec à Trois Rivières*
C.T. Marshall, *University of Aberdeen*
P. Moran, *Universidad de Vigo*
J. Post, *University of Calgary*
Y.T. Prairie, *Université du Québec à Montréal*
T.J. Quinn II, *University of Alaska – Fairbanks*
C.W Ramcharan, *Laurentian University*
J.S. Richardson, *The University of British Columbia*
M.-J. Rochet, *French Research Institute for Exploitation of the Sea, Nantes*
K. Rose, *Louisiana State University*
J. Rosenfeld, *British Columbia Ministry of Fisheries*
B. Sainte-Marie, *Fisheries and Oceans Canada, Mont-Joli*
R.E.H. Smith, *University of Waterloo*
S.J. Smith, *Fisheries and Oceans Canada, Dartmouth*
W.G. Sprules, *University of Toronto*
E.B. Taylor, *The University of British Columbia*
W. Tonn, *University of Alberta*
M. Trudel, *Fisheries and Oceans Canada, Nanaimo*
A. Vasemägi, *University of Turku*
C.J. Walters, *The University of British Columbia*
G. Weyhenmeyer, *Uppsala University*

NRC Research Press / NRC Research Press

Fax / télécopieur : 613-952-7656; e-mail / courriel : pubs@nrcresearchpress.com; URL / URL : nrcresearchpress.com

Editor-in-Chief / Directeur de la publication **Bruce P. Dancik**
Deputy Editor-in-Chief / Sous-directrice de la publication **Cindy E. Prescott**
Executive Director / Directeur général **Cameron D. Macdonald**

Director, Business Strategy and Development / Directeur, Stratégie et développement des affaires **Mike Boroczki**
Manager, Business Development / Gestionnaire du développement **Peter D'Amico**
Marketing and Sales Coordinator / Coordonnatrice des ventes et du marketing **Judy Letourneau** (613-993-0151)
Client Services Representative / Représentante des services à la clientèle **Eileen Evans-Nantais** (613-990-7873)

Director, Publishing Operations / Directrice, Services d'édition **Suzanne Kettley**
Managing Editor / Directrice de la rédaction **Cecily M. Pearson** (613-993-9099)
Scientific Publishing Editors / Réviseurs de publications scientifiques **Thad Costigan, Tina Wilston**
Publishing Assistant / Adjointe à l'édition **Diana Leck**

Acting Production Manager / Gestionnaire de la production par intérim **Cory Klinkenberg**
e-Publishing Technician / Technicien à l'édition électronique **Jeffrey Wisking**
Graphics Technician / Technicien en graphisme **Leslie Cameron**
Web Technician / Technicien Web **Dennis Cafferty**

NRC Research Press accepts no responsibility for statements or opinions expressed by a contributor. Acceptance of an advertisement, announcement, or other material does not imply endorsement by either the Journal's Editors or NRC Research Press.

© 2011 Canadian Science Publishing or its licensors

As of 2009, copyright in all articles in NRC Research Press journals remains with the authors. All articles published previously are copyright Canadian Science Publishing (which operates as NRC Research Press) or its licensors.

No part of the NRC Research Press journals may be reproduced, stored, or transmitted in any form or by any means, without the written permission of the publisher, except as stated below.

Under the Canadian *Copyright Act*, individuals may download or print single copies of articles for personal research or study. Any person may reproduce short excerpts from articles in the journals for any purpose that respects the moral rights of authors, provided that the source is fully acknowledged. As a courtesy, the consent of authors of such material should be obtained directly from the author.

Authorization to reproduce items for other than personal research or study, as stated above, may be obtained by clicking on the "Reprints and Permissions" link in the table of contents of the electronic article in question or via Access Copyright (www.accesscopyright.ca).

NRC Research Press also extends certain additional rights to authors.

The above rights do not extend to copying or reproduction for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale. For such copying or reproduction, arrangements must be made with NRC Research Press, Ottawa, ON K1A 0R6, Canada.

Subscription information

The *Canadian Journal of Fisheries and Aquatic Sciences* (ISSN 0706-652X) is published monthly by NRC Research Press. The 2011 subscription rates are as follows. Institutional: electronic $1240; print, $1490; electronic + print, $1865. Personal: electronic, $320, print + electronic, $430. For all orders outside Canada, rates are in U.S. dollars. Print subscriptions include second class postage. For air mail add US$204 to the foreign rate. Canadian customers please include GST (business no. 81942 4656 RT0001). Subscriptions are entered for the calendar year. Remittances should be made payable to Canadian Science Publishing. Price for regular single issues: institutional, $150.00; personal, $50.00. Supplements will be priced separately. Special rates apply to members of Canadian scientific and engineering societies if they subscribe through their respective societies.

Issues of the *Canadian Journal of Fisheries and Aquatic Sciences* are available on the Web. Pricing information can be found at nrcresearchpress.com/page/librarians/subscriptions/pricing.

There are no refunds on cancelled subscriptions once delivery has begun. All claims must be submitted within 12 months of the publication date. The publisher expects to supply missing issues only when losses have occurred in transit and when the reserve stock will permit. Subscriptions, renewals, requests for single or back issues, and all remittances should be sent to Canadian Science Publishing, NRC Research Press, Subscription Office, P.O. Box 830399, Birmingham, AL 35283-0399, USA (telephone: 1-800-633-4931 in North America or 1-800-852-7404 outside North America; fax: 205-995-1588).

Microform backfiles are available from NA Publishing Inc., P.O. Box 998, Ann Arbor, MI 48106-0998, U.S.A., (734) 302-6500. www.napublishing.com.

NRC Research Press ne se tient en aucune façon responsable des opinions exprimées par un collaborateur ou des déclarations que pourrait faire ce dernier. La publication d'annonces publicitaires, d'avis et autres n'implique pas l'endossement de leur contenu par les rédacteurs en chef de la Revue ou NRC Research Press.

© 2011 Éditions Sciences Canada ou ses concédants de licence

Depuis 2009, les auteurs conservent le droit d'auteur de tout article qu'ils publient dans les revues de NRC Research Press. Éditions Sciences Canada (qui œuvre sous la dénomination commerciale NRC Research Press) ou leurs concédants de licence, quant à eux, se réservent les droits d'auteur sur tous les articles précédemment publiés à NRC Research Press.

Il est interdit de reproduire, de sauvegarder ou de transmettre toute partie des revues de NRC Research Press dans quelque format ou de quelque façon que ce soit sans le consentement écrit de l'éditeur, sauf dans les cas énoncés ci-après.

Aux termes de la *Loi sur le droit d'auteur* du Canada, les particuliers peuvent télécharger ou imprimer des copies individuelles d'articles pour leurs études ou leurs recherches personnelles. Toute personne peut reproduire de courts extraits des articles d'une revue pourvu que les droits moraux de l'auteur soient respectés et que la source soit clairement indiquée. Il convient de demander directement à l'auteur son consentement.

On peut obtenir l'autorisation de reproduire du contenu à d'autres fins que l'usage personnel (tel que décrit ci-dessus), en cliquant sur le lien « Réimpressions et autorisations » dans la table des matières de l'article électronique en question ou par l'intermédiaire d'Access Copyright (www.accesscopyright.ca).

NRC Research Press accorde aussi des privilèges supplémentaires aux auteurs.

Les droits ci-dessus ne s'appliquent pas à la reproduction à grande échelle aux fins de distribution générale, de publicité ou de promotion, de compilation de nouveaux ouvrages collectifs ou de revente, par exemple. Les droits de reproduction de ce genre doivent faire l'objet d'une entente particulière avec NRC Research Press, Ottawa, ON K1A 0R6, Canada.

Renseignements sur l'abonnement

Journal canadien des sciences halieutiques et aquatiques (ISSN 0706-652X) est une publication mensuelle de NRC Research Press. En 2011, les tarifs d'abonnement pour les organismes sont les suivants : 1240 $ pour la version électronique; 1490 $ pour l'imprimé; 1865 $ pour les deux versions. Pour leur part, les particuliers paient les tarifs suivants : 320 $ pour la version électronique; 430 $ pour la version électronique et l'imprimé. Les tarifs des commandes provenant de l'extérieur du Canada sont en dollars des États-Unis. L'abonnement à l'imprimé comprend le tarif du courrier de deuxième classe. L'expédition par avion exige des frais de 204 $US en sus du prix d'abonnement à l'étranger. Les clients du Canada doivent ajouter la TPS (numéro d'entreprise 81942 4656 RT0001). Les abonnements sont pour l'année civile. Tout paiement doit être adressé aux Éditions sciences Canada. Le prix par numéro régulier est de 150,00 $ pour les organismes et de 50,00 $ pour les particuliers. Le prix des hors-série varie. Les membres des sociétés canadiennes de sciences et de génie bénéficient de taux préférentiels en s'abonnant via leurs sociétés respectives.

Tous les numéros de Journal canadien des sciences halieutiques et aquatiques sont accessibles sur le Web. On trouvera des renseignements sur les tarifs au nrcresearchpress.com/page/librarians/subscriptions/pricing.

Aucun remboursement ne sera accordé, ni aucune annulation acceptée, une fois que les livraisons auront commencé. Toute réclamation doit être présentée dans les 12 mois suivant la date de publication. L'éditeur ne fournira de numéros manquants que si le document a été perdu pendant l'expédition et que si les réserves le permettent. Pour un abonnement ou un renouvellement, pour une demande de numéro (actuel ou ancien), ainsi que pour une réclamation, adressez-vous au Bureau des abonnements, Éditions Sciences Canada, NRC Research Press, P.O. Box 830399, Birmingham, AL 35283-0399, É.-U. (téléphone : 1-800-633-4931, en Amérique du Nord, ou 1-800-852-7404, ailleurs; télécopieur : 205-995-1588).

On peut obtenir les archives sur microforme auprès de NA Publishing, Inc., P.O. Box 998, Ann Arbor, MI 48106-0998, U.S.A., (734) 302-6500, www.napublishing.com.

Why does egg size of salmonids increase with the mean size of population spawning gravels?

Njal Rollinson and Jeffrey A. Hutchings

Abstract: Population mean egg size of salmonids increases with the mean size of gravels in which a population spawns. A long-standing hypothesis is that large larvae cannot navigate small-gravel interstices, so mothers must decrease per-offspring investment when spawning gravels are small. We manipulated the size of incubation gravels and egg size of Atlantic salmon (*Salmo salar*) to test whether size-related entombment of larvae occurs. We find little evidence of size-related entombment, but we find evidence that gravel size does not affect all larval sizes equally. Larger larvae emerge from small gravels before development is complete and with a visible yolk sac, possibly due to oxygen limitation in small gravels. Smaller larvae always complete development in the gravel and emerge without a yolk sac. Although growth and survival may increase with juvenile size following yolk-sac absorption, juveniles with yolk sacs may fare worse when depredation rates are high. The egg size – gravel size correlation may therefore reflect increased postemergence mortality among larger offspring in small-gravel environments. Alternatively, compaction stress in fine gravels coupled with size asymmetries in larval strength may have caused the patterns that we observed, in which case our data may not help explain the egg size – gravel size correlation.

Résumé : La taille moyenne des oeufs d'une population de salmonidés augmente en fonction de la taille moyenne du gravier dans lequel cette population fraie. Une hypothèse de longue date veut que les larves de grande taille ne peuvent pas se déplacer dans les interstices de gravier fin et que les mères doivent réduire leur investissement par rejeton lorsque le gravier de fraie est fin. Nous avons manipulé la taille du gravier d'incubation et la taille des œufs chez des saumons atlantiques (*Salmo salar*) afin de tester s'il se produit un ensevelissement des larves en fonction de la taille. Nous trouvons peu d'indications d'un ensevelissement relié à la taille, mais il y a des preuves que la taille du gravier n'affecte pas toutes les tailles de larves de même manière. Les larves plus grandes émergent des graviers fins avant la fin de leur développement et avec un sac vitellin bien visible, peut-être à cause de la limitation de l'oxygène dans les graviers fins. Les larves plus petites complètent toujours leur développement dans le gravier et émergent sans sac vitellin. Bien que la croissance et la survie puissent augmenter en fonction de la taille des jeunes après la absorption du sac vitellin, les jeunes avec sac vitellin peuvent réussir moins bien lorsque les taux de prédation sont élevés. La corrélation entre la taille des œufs et la taille du gravier peut donc refléter une mortalité accrue après l'émergence chez les rejetons de plus grande taille dans les environnements de gravier fin. Par ailleurs, le stress de compaction dans les graviers fins combiné aux asymétries reliées à la taille dans les forces des larves peuvent avoir causé les patrons que nous avons observés; dans ce cas, nos données peuvent ne pas aider à expliquer la corrélation entre la taille des œufs et la taille du gravier.

[Traduit par la Rédaction]

Introduction

As mothers are expected to allocate the energy available for reproduction into offspring of an optimal size (Smith and Fretwell 1974), variation in offspring size among populations is often assumed to reflect a matching of maternal provisioning strategies to a particular environment (e.g., Gregersen et al. 2008). Indeed, interspecific variation in egg size may reflect broad patterns of canalization or adaptive plasticity in response to local selection pressure on eggs, embryos, and offspring (Berven and Gill 1983; Fox et al. 1997; Bashey 2006). Although a consistent difference among populations in the strength or direction of selection on offspring size can theoretically lead to adaptive divergence in per-offspring investment (Smith and Fretwell 1974; Allen et al. 2008; Marshall and Keough 2008), opposing patterns of selection at different stages of offspring development may also provide a framework in which adaptive interpopulation variation in offspring size can be understood (Hendry et al. 2001). For example, when offspring fitness is negatively related to size during the egg stage and positively related to size during the juvenile stage (e.g., Congdon and Gibbons 1987; Sinervo and Licht 1991), optimal offspring size becomes a function of the relative strength of selection on offspring size in the different life stages (Hendry et al. 2001). Although this mechanism can likely account for population divergence in egg size (e.g., Parciak 2002), few studies have explicitly examined its role in maintaining interspecific egg-size variation.

Received 22 September 2010. Accepted 22 April 2011. Published at www.nrcresearchpress.com/cjfas on 27 July 2011.
J22032

Paper handled by Associate Editor Ian Fleming.

N. Rollinson and J.A. Hutchings. Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada.

Corresponding author: Njal Rollinson (e-mail: njal.rollinson@gmail.com).

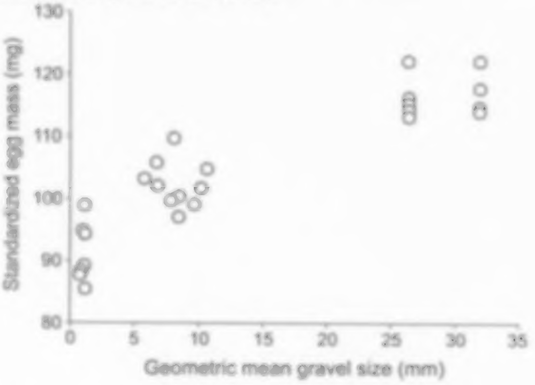
Can. J. Fish. Aquat. Sci. **68**: 1307–1315 (2011) doi:10.1139/F2011-069 Published by NRC Research Press

Salmonids are largely autumnal spawners that deposit their eggs into riverine or lacustrine gravels. Embryos develop over winter and hatch into yolk-sac larvae (or "alevins") in the following spring. Once yolk-sac absorption is complete, individuals emerge from the gravel as juveniles (or "fry"), and in some species, they establish feeding territories near their natal area (e.g., Bachman 1984). Size asymmetries at the juvenile stage (Hutchings 1991; Einum and Fleming 2000*a*, 2000*b*) and at the embryo or larval stage (Einum et al. 2002) can affect maternal and offspring fitness in salmonids such that this group has stimulated a wealth of research on offspring size–number strategies (reviewed in Einum et al. 2004).

Famously, a clear, positive correlation between population-specific spawning gravel size and population mean egg size has been observed among 21 Alaskan populations of sockeye salmon (*Oncorhynchus nerka*) (Quinn et al. 1995; Quinn 2005; Fig. 1). This pattern may occur because small incubation gravels represent (or are correlated with) a relatively benign selective environment in which variation in offspring size is not correlated with variation in offspring fitness. Under these conditions, maternal fitness is indeed maximized by producing many, poorly provisioned offspring (Smith and Fretwell 1974). It seems unlikely, however, that small gravels represent a benign selective environment, as small incubation gravels are generally associated with low egg-to-juvenile survival, which in turn has been attributed to low oxygen availability and small-gravel interstices (Phillips et al. 1975; Witzel and MacCrimmon 1981; Olsson and Persson 1986). The egg size – gravel size correlation may instead result from a decrease in offspring fitness with increasing egg size in small gravels. Only small larvae may be able to navigate the small pore spaces in finer gravels, whereas larger larvae may become entombed in the nest cavity. However, as the size of incubation gravels increases, there may be no size-related advantage during the embryo or larval stage, and given that larger juveniles generally exhibit greater fitness in the wild (Einum and Fleming 2000*a*, 2000*b*), a larger egg size may maximize maternal fitness when gravel sizes are large. Although this widely discussed hypothesis has never been explicitly tested (Witzel and MacCrimmon 1983; Quinn et al. 1995; Quinn 2005), it suggests that opposing patterns of selection at different life stages has led to a divergence in optimal offspring sizes among environments (Hendry et al. 2001).

The maximum difference between the smallest and largest population mean egg size in the original sockeye salmon populations was 25.6% (Quinn et al. 1995). The magnitude of this difference is indeed sufficient to warrant an investigation of adaptive hypotheses, as limited data suggest that a difference in egg size of 14%–30% is typical when reasonable evidence of adaptive egg-size plasticity exists (summarized by Fischer et al. 2011). On the other hand, although variation in egg size can be related to proximate factors such as variation in the offspring selective environment (e.g., Fox et al. 1997), it can also be traced to ultimate factors such as the rate at which a mother can provision primary oocytes (Sakai and Harada 2001). The point is that variation in mean egg size among environments does not necessarily mean variation in optimal offspring size. Manipulative experiments are necessary to test whether interpopulation egg-size variation reflects an adaptive response to local environments.

Fig. 1. The relationship between population mean egg size of sockeye salmon (*Oncorhynchus nerka*) and geometric mean size of spawning gravels. Multiple points for the same gravel size are samples from the same population in different years. Egg mass is adjusted to a common maternal body size of 450 mm fork length. Modified from Quinn et al. (1995).



Here, we manipulate incubation environments of larval Atlantic salmon (*Salmo salar*) to test whether large embryos and larvae exhibit poor survival in small gravels because of size-related larval entombment. Assuming that size-related entombment is a function of larval size per se, we expect that larvae hatching from relatively large embryos will experience lower survival than larvae hatching from smaller embryos in fine gravels, but not in larger gravels. If large larvae have difficulty navigating the small interstices of fine gravels, then we also expect that they will use more energy during emergence from these gravels. Thus, we predict further that in smaller gravels, large juveniles surviving to emergence will have a lower specific energy content (in joules per gram of tissue) compared with smaller juveniles, but no difference in specific energy will be observed between large and small individuals that have emerged from a large-gravel substrate.

Materials and methods

Atlantic salmon were reared to maturity at the Coldbrook Biodiversity Facility in Coldbrook, Nova Scotia, Canada. As part of a larger study on local adaptation in Atlantic salmon, multigenerational crosses were generated from 49 females and 54 males on 31 October 2008 and 4 November 2008; between four and eight males were crossed with a given female (for details, see Houde et al. 2011). Embryos were incubated at ambient temperatures in incubation trays within a single incubation trough at Coldbrook until the eyed stage. Embryos were then transferred to the Aquatron Facility at Dalhousie University where they were kept in plastic containers (Lee's Kritter Keepers, Lee's Aquarium & Pet Products, San Marcos, California) until the beginning of the experiment. Each container measured 27.6 × 17.0 × 20.3 cm but was modified to accommodate two families in 13.8 ×17.0 × 20.3 cm sections, and each container was perforated to allow water flow. Up to three containers (up to six families) occupied one 70 cm circular flow tank, and 60 flow tanks were used in total. Water levels were maintained so that embryos were submerged under 7 cm of water, and water volume was roughly 26.9 L·tank^{-1}. Flow into each tank was 5–10 mL·s^{-1}, and aeration originated from the middle of each tank, which promoted homogeneity in water quality. Ambient temperatures were maintained throughout incubation (mean ± standard deviation (SD): 3.1 ± 2.3 °C at Coldbrook; 5.2 ± 1.6 °C in the Aquatron). Percentage development was estimated from the sum of daily development, where daily development is estimated by Kane (1988) as

$$(1) \qquad \ln(y) = 6.003\, e^{(-0.0307 \times T)}$$

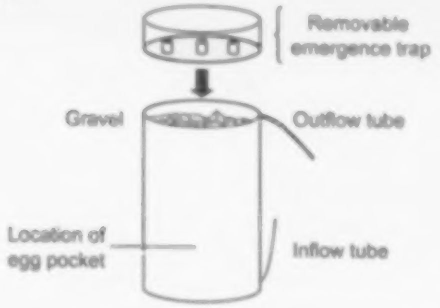
where "e" is the base of the natural logarithm, and T is the mean daily incubation temperature in degrees Celsius. Each daily value (y) was divided into 100 and then summed across the incubation period to obtain percentage development (juveniles are expected to emerge from the gravel at 100% development).

We constructed water-tight containers designed to simulate natural egg pockets within salmon redds (Fig. 2). Containers were constructed from 15.2 cm diameter polyvinyl chloride (PVC) piping cut into 30 cm pieces. The base of each container was fixed with a plastic, water-tight cap, but the top was left open. Dechlorinated water was fed into the container through 0.25 cm tubing fixed in a small hole that was drilled 2 cm above base of each container. Water flowed upward through the container and then out the top of the container through a 0.75 cm tube fixed to the lip. Water flow through each container was maintained at 26.1 ± 3.1 mL·s^{-1}. Emergence traps were created by cutting 1 cm diameter tubing into 0.75 cm sections and then fixing those sections into similar-sized perforations in transparent, circular, 15 × 5 cm plastic trays. The trays were then secured in the top of each container.

The experiment was a 3 × 3 factorial design with three levels of egg size and three levels of gravel size, with three replicates per treatment (3 egg sizes × 3 gravel sizes × 3 replicates = 27 replicates in total). Gravel was purchased from Conrad Bros Ltd. (Dartmouth, Nova Scotia) and sieved into three size classes. Mean ± SD diameter of 75 randomly selected pebbles (measured along the median or "B" axis) was 28.2 ± 3.4 mm (large gravel), 11.7 ± 1.6 mm (medium gravel), and 8.9 ± 1.6 mm (small gravel). The mean, median, and geometric mean gravel size was very similar for each size class. The range of geometric mean gravel sizes identified by Quinn et al. (1995) was 1.6–77.8 mm (pebble count method) and 0.9–32.1 mm (bulk samples).

Initial mean egg mass for each of the 49 females was estimated from a sample of 12–20 unfertilized eggs collected in Coldbrook. Based on these estimates, we identified six females that produced "small" eggs, six that produced "medium" eggs, and five that produced "large" eggs; the females that contributed eggs to each egg size class came from a mix of three different populations and hybrids thereof (Table 1). One female in each of these size classes was spawned on 4 November 2008, and the rest were spawned on 31 October 2008. On 10 and 11 March 2009, embryos were selected from relevant families (Table 1) and allocated evenly to the appropriate egg size class treatment. Allocation proceeded as in the following example: 19 large embryos may have been collected from each of four families (76 embryos in total) that share a common mother. In this case, two embryos per family (eight embryos in total) would then have been allocated to each of the nine large-egg replicates, with four of the 76 embryos remaining unused. One or two of the unused embryos from each family were retained and weighed to the nearest 0.1 mg to obtain embryo mass (Table 1), the others were discarded. Estimated mean embryo mass (± standard error, SE) allocated to each small-, medium-, and large-egg replicate was 69.5 ± 1.1 mg, 103.6 ± 1.5 mg, and 138.0 ± 2.6 mg, respectively, thus exceeding the range of population mean egg sizes identified by Quinn et al. (1995), which were between 84.5 ± 2.7 mg and 113.5 ± 1.2 mg. The small- and large-egg replicates each contained 44 embryos, medium-egg replicates contained 45 embryos, and all embryos were buried under 23 cm of gravel.

Fig. 2. Artificial salmon egg pocket (nest replicate) used in the present study.



A natural photoperiod was initiated on 31 March 2009. Juvenile emergence occurred between 13 May and 18 June 2009. When juveniles were detected in an emergence trap, they were captured with a turkey baster, overanaesthetized in 0.1 mg·L^{-1} tricaine methanesulphonate, rinsed in freshwater, blotted dry, weighed to the nearest 0.1 mg, and measured to the nearest millimetre (standard length) using calipers. We also noted the presence or absence of a yolk sac, as the mean percentage yolk content of juveniles emerging from a replicate is highly correlated with the percentage of individuals emerging with a visible yolk sac ($r^2 = 0.971$, $n = 12$ replicates, $P < 0.001$; N. Rollinson and J.A. Hutchings, unpublished data). Juveniles were then frozen in individual 3 mL plastic tubes at −4 °C. All replicates were excavated on 24 June 2009, which is six days after the last juvenile had emerged, and the remaining live and dead alevins were counted.

Between 12 and 15 July 2010, we combusted a subsample of juveniles from each replicate to estimate their energetic content. First, the median day of emergence was calculated separately for each replicate. Then, three (n = 4 replicates) or four (n = 23 replicates) juveniles that emerged on the median day of emergence, or as close as possible to the median day of emergence, were vacuum dried for 18 h using a Labconco 4.5 L freeze-dryer (n = 104 juveniles dried in total). Dry mass was then measured to the nearest 0.1 mg. To estimate energetic content of tissue, the subsample was homogenized and then combusted in a Parr 1266 semimicro oxygen bomb calorimeter. Each subsample was compressed to a 3 mm diameter pellet, and pellets were combusted using 1 of 2 Parr 1107 micro-bombs, as described by Michaud and Taggart (2007).

Table 1. Summary of embryo allocation to the experiment.

♀ ID	Egg size	Maternal cross	Embryo mass (mg)	SE	No. of embryos	No. of families
1	Small	Stewiacke	66.7	2.8	12	4
2	Small	Stewiacke	73.9	1.5	12	4
3	Small	Stewiacke	70.1	2.6	4	4
4	Small	Great Village	70.3	1.6	5	5
5	Small	Great Village	67.6	2.9	6	4
6	Small	Stewiacke	61.5	1.0	5	5
7	Medium	Stewiacke	107.7	0.9	9	4
8	Medium	Economy	100.1	4.3	8	5
9	Medium	Great Village	103.5	1.2	6	5
10	Medium	Economy	103.6	3.9	7	3
11	Medium	Eco × Stew	108.2	1.9	8	2
12	Medium	Grv × Stew	98.5	3.4	7	2
13	Large	Great Village	138.5	2.3	4	4
14	Large	Eco × Stew	137.4	1.0	8	2
15	Large	Eco × Stew	150.9	0.7	10	2
16	Large	Great Village	149.4	1.2	11	6
17	Large	Stewiacke	130.0	1.9	11	6

Note: "Maternal cross" reflects the genetic background of the female (Stewiacke River (Stew); Great Village River (Grv); Economy River (Eco)). "No. of embryos" is the number allocated to each relevant replicate from a given female. "No. of families" is the number of half-sib families (of embryos) allocated to the experiment from a given female. SE, standard error.

Factorial analyses of variance (ANOVAs) and Tukey post hoc tests were performed in Minitab v. 15.1.3 (Minitab Inc., State College, Pennsylvania). Weighted fits were used when cell means were computed from unequal sample sizes, and proportional data were logit(x + 0.025)-transformed before analysis.

Results

We found a tight relationship between wet and dry mass of the 104 dried juveniles (dry mass, mg = 0.188(wet mass, mg) – 3.23, $r^2 = 0.983$, $P < 0.001$), so all values of juvenile mass reported below are dry mass values predicted from wet mass. Over the course of the experiment, 10 juveniles escaped from their replicates and were found in the overflow sink. They could not be assigned back to their original container, but they varied in size (range, 11.5–52.6 mg; mean ± SD, 21.3 ± 4.2 mg), which indicates that they did not come from a single replicate.

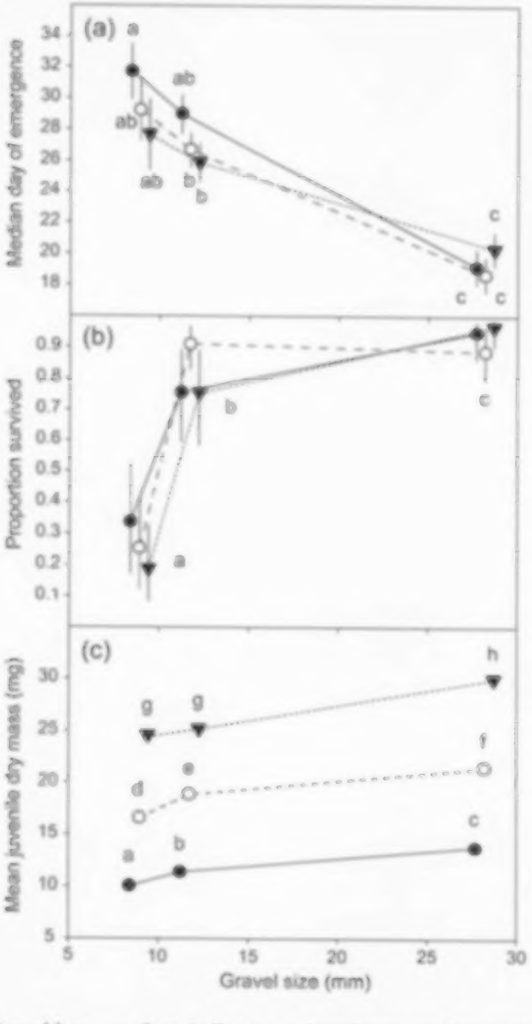
Emergence began on 13 May 2009 at 102.8% development and finished on 18 June 2009 at 164.8% development. We detected significant effects of egg size ($F_{[2,18]} = 5.8$, $P = 0.011$), gravel size ($F_{[2,18]} = 142.9$, $P < 0.001$), and their interaction ($F_{[4,18]} = 3.3$, $P = 0.032$) on percentage development at emergence. Similarly, we detected significant effects of egg size ($F_{[2,18]} = 6.4$, $P = 0.008$) and gravel size ($F_{[2,18]} = 185.5$, $P < 0.001$) on median day of emergence, as well as a gravel size – egg size interaction ($F_{[4,18]} = 4.3$, $P = 0.013$). Both estimates of emergence timing indicated that individuals from small embryos emerged relatively late in finer gravels, but there was no difference among egg size treatments in emergence time in large gravel (Fig. 3*a*).

Survival increased with increasing gravel size ($F_{[2,18]} = 66.5$, $P < 0.001$), but survival was not related to egg size ($F_{[2,18]} = 0.52$, $P = 0.60$), and the egg size – gravel size interaction was not significant ($F_{[4,18]} = 2.6$, $P = 0.070$; Fig. 3*b*). Dry mass at emergence was significantly affected by egg size ($F_{[2,18]} = 3696.6$, $P < 0.001$) and gravel size ($F_{[2,18]} = 487.6$, $P < 0.001$), and the egg size – gravel size interaction was significant ($F_{[4,18]} = 19.5$, $P < 0.001$). For large-egg treatments, juvenile mass did not differ between medium-gravel and small-gravel treatments, apparently because juveniles were smaller than expected in the large-egg – medium-gravel treatment. Differences in mass were apparent for medium- and small-egg treatments at each level of gravel size (Fig. 3*c*).

Specific energy of juveniles averaged 21.86 ± 0.12 $kJ{\cdot}g^{-1}$ across all treatments. We detected a significant effect of egg size ($F_{[2,18]} = 4.8$, $P = 0.021$) and an egg size – gravel interaction ($F_{[4,18]} = 3.4$, $P = 0.031$). Specific energy was significantly greater in the large-egg – small-gravel treatment compared with the small-egg – small-gravel and small-egg – medium-gravel treatments, but there was no difference among treatments in large gravel (Fig. 4*a*). To obtain total energy of juveniles (in joules), we multiplied the specific energy value obtained from each replicate by the mean dry mass of juveniles emerging from that replicate. In the subsequent ANOVA, we observed significant effects of egg size ($F_{[2,18]} = 1166.5$, $P < 0.001$), and gravel size ($F_{[2,18]} = 145.4$, $P < 0.001$), and the egg size – gravel size term was significant ($F_{[4,18]} = 4.1$, $P = 0.015$). Total energy increased with increasing gravel size for the medium-egg treatments, but total energy was not different among the two smaller gravel sizes for both small and large eggs. Within egg-size treatments, total energy was always greatest in large gravel (Fig. 4*b*).

Individuals from small embryos never emerged with a yolk sac at any level of gravel size, whereas many individuals from medium and large embryos emerged from small gravels with a yolk sac (Fig. 4*c*). Accordingly, the proportion of fry emerging with a yolk sac ("proportion with yolk") was zero-heavy and not normally distributed (Kolmogorov–Smirnov test statistic = 0.352, $P < 0.001$). These data pose a statisti-

Fig. 3. (*a*) Median day of juvenile emergence for each level of egg size and gravel. Note that post-hoc tests show an identical pattern of significance when emergence time is expressed as percentage development. (*b*) Embryo-to-juvenile survival as a function of egg size and gravel size. Proportions are back-transformed from logits. Note that only the main effect of gravel was significant, and that the single letter at each level of gravel size denotes differences for the main effect of gravel size. (*c*) Mean dry mass of juveniles. Solid circles, small eggs; open circles, medium eggs; solid triangles, large eggs. Letters denote significant differences after a Tukey's honestly significant difference (HSD) test; means are least-squares means and error bars are 95% confidence intervals.

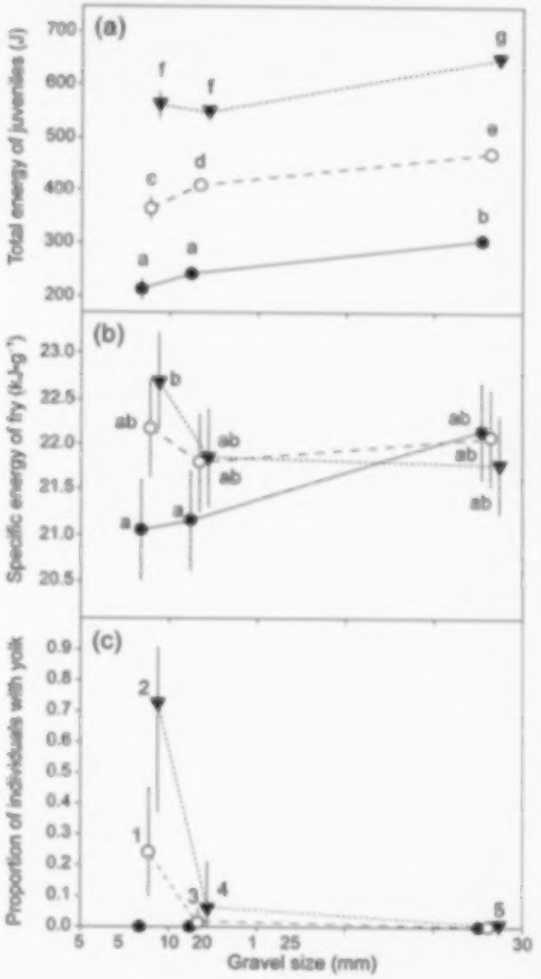


cal problem, as they indicate an invariance of "proportion with yolk" in the small-egg level of the egg-size factor. Most statistical tests assume that data are drawn from populations with the same distribution of data, even if the distribution is non-normal. In our case, the proportion of individuals emerging with a yolk sac from small-egg treatments was always zero, and hence distribution-free, so a more appropriate approach is to calculate the probability of drawing a zero from a population with a mean and standard deviation equal to those observed in the other treatments. To evaluate whether treatments with a nonzero mean were significantly greater than zero, we used one-tailed z tests,

$$z = (x_i - \bar{x}) \times \sigma^{-1} \tag{2}$$

where x_i is 0, $\bar{x}$ is the weighted mean of a given treatment, and σ is the weighted standard deviation (Zar 1984, p. 84). We also applied a Bonferroni correction to account for multiple paired comparisons (five tests were performed, so α =

Fig. 4. (*a*) Least squares mean total energy of juveniles. (*b*) Least squares mean specific energy of juveniles. (*c*) Weighted mean proportion of individuals that emerged from the gravel with a visible yolk sac as a function of gravel size and egg size. Data are back-transformed from logits. Values identify one-tailed z tests (α = 0.01) performed on logit-transformed data, which evaluated whether nonzero means were significantly greater than zero (1, z = –2.72, P = 0.003; 2, z = –3.11, P = 0.0009; 3, z = –0.57, P = 0.28; 4, z = –1.09, P = 0.14; 5, z = –0.56, P = 0.28). Solid circles, small eggs; open circles, medium eggs; solid triangles, large eggs. Letters denote significant differences after a Tukey's honestly significant difference (HSD) test; error bars are 95% confidence intervals.



0.01). Weighted mean proportion of individuals emerging with a yolk sac was greater than zero for the large-egg – small-gravel treatment (logit($\bar{x}$+ 0.025) = 1.20, logit(σ + 0.025) = 1.56, logit(x_i + 0.025) = –3.66, z = –3.11, α = 0.01, P = 0.0009) and for the medium-egg – small-gravel treatment ($\bar{x}$= –0.933, σ = 1.00, x_i = –3.66, z = –2.72, α = 0.01, P = 0.003), whereas other treatments were not greater than zero (Fig. 4*c*). This suggests an egg size – gravel size interaction, as there is no difference among levels of egg size in larger gravels, but "proportion with yolk" increases in juveniles from medium and large eggs when gravel size is small (Fig. 4*c*). Finally, "proportion with yolk" correlated positively with specific energy (Spearman's rho = 0.516, n = 27, P = 0.006), indicating that juveniles emerging with yolk sacs had a higher specific energy.

Six days after the last juvenile emerged from the gravel (24 June 2009), we assumed that all larvae surviving within the replicates were unable to escape (entombed) and excavated all replicates. We analyzed these data to provide insight into the mechanism(s) of mortality within the gravel (e.g., oxygen limitation versus entombment). All live individuals detected were emaciated, suggesting entombment. Only four live individuals were found in the nine medium-gravel replicates, all four of which were in two large-egg replicates. No live individuals were found in any of the large-gravel replicates. Hence, we analyzed entombment data only within small gravels using a one-way ANOVA, where we tested the effect of egg size. The percentage of live individuals entombed in small-, medium-, and large-egg treatments was 11.1%, 12.3%, and 23.7%, respectively, and these values are inversely related to survival to emergence in small gravels (Fig. 3*b*). The one-way ANOVA was not significant ($F_{[2,6]}$ = 2.5, P = 0.16), however, which indicates that survival after entombment was not related to egg size in small gravel.

Dead individuals were too decomposed to determine stage-at-death (i.e., yolk-sac vs. non-yolk-sac larvae). Not surprisingly, the percentage of dead individuals found after excavation was higher in small gravel ($F_{[1,12]}$ = 53.4, P < 0.001) but unaffected by egg size ($F_{[2,12]}$ = 2.2, P = 0.15) or their interaction ($F_{[2,12]}$ = 1.4, P = 0.29). Note that no dead individuals were detected in the large-gravel replicates, so the large-gravel level of the gravel size factor was not included in the preceding analysis.

Discussion

In the present study, we test whether interpopulation variation in egg size could be maintained by size-specific survival of embryos or larvae in particular incubation substrates. We observed that the effect of egg size on offspring phenotypes differed markedly among incubation substrates: the egg size – gravel size interaction term was significant in four of five ANOVAs. Although our survival data suggest that individuals from small embryos fared similarly in both small and medium gravel (which cannot be said of individuals from medium and large embryos), the relevant interaction term for this effect was not significant (P = 0.07). Our data do not, therefore, support our focal hypothesis, but they instead intimate the possibility that a novel mechanism may effect divergent selection on egg size among populations of salmonids. Below, we re-evaluate traditional hypotheses for the egg size – gravel size correlation and examine whether our data can help explain this correlation.

First, we observed interactions among egg size and specific energy, day of emergence, and percentage of juveniles emerging with a yolk sac. These interactions are not independent, as both specific energy and emergence time may correlate with percentage yolk (developmental stage) within gravel size fractions. These interactions broadly reflect a positive correlation between initial egg size and the proportion of juveniles emerging with yolk in smaller gravels and a lack of this correlation in larger gravels. Moreover, small larvae emerged relatively late in small gravel, but not in larger gravels, and this is reflected in the lower specific energy (or percentage yolk) of small larvae in small gravels. Overall, these patterns suggest that relatively large larvae emerge prematurely in small incubation gravels, whereas small larvae delay emergence until yolk absorption is complete. A common observation is indeed that premature emergence occurs when incubation gravels are small (Phillips et al. 1975; Witzel and MacCrimmon 1983; Olsson and Persson 1986) and that larger larvae take longer to absorb their larger yolk sacs (Killeen et al. 1999). Our findings are novel, however, in that premature emergence in stressful environments is much more common among larger larvae.

Flow in artificial incubators can be largely confined to the smooth incubator walls when gravel size is small (Rose and Rizk 1949; Witzel and MacCrimmon 1983), and given that larval mobility is restricted in smaller gravels (Dill and Northcote 1970), oxygen stress may have occurred in the present study. Assuming zero mortality in our replicates, estimated total oxygen consumption for small-, medium-, and large-egg replicates was 0.0853, 0.141, and 0.191 mg·h^{-1}, respectively, at the eyed embryo stage, and 0.0985, 0.261, and 0.479 mg·h^{-1}, respectively, just after hatching (i.e., the beginning of the larval stage) (calculations following Rombough 2007). For large-egg replicates, this suggests an increase of 60.1% in oxygen consumption from the embryo stage to the larva stage compared with a 13.4% increase for small-egg replicates. Of course, we did not detect a size-related difference in survival to emergence or in the number of larvae surviving while entombed in small gravels at the end of the experiment, so if a size-related oxygen effect occurred, it was likely sublethal.

Yet it is unlikely that size-related patterns of emergence reflect different hypoxic tolerances of small and large embryos. Small embryos have indeed been long expected to fare better in low-oxygen environments because of larger ratios of surface area to volume, which was presumed to translate into more favourable rates of gas exchange (Krogh 1959); however, this supposition has been discredited. Although geometric constraints dictate that egg and embryo surface areas increase at a rate of (embryo mass)$^{0.67}$, the rate of metabolic expansion is much slower (b = 0.44 (Einum et al. 2002); b = 0.30 (Rombough 2007)) such that larger embryos actually have a greater surface area for gas exchange relative to metabolic oxygen demand. Despite this difference, embryo size has no effect on hypoxic tolerance during the embryo stage, so it is unlikely that embryo surface area is a major factor limiting oxygen uptake (Rombough 2007; but see Einum et al. 2002). Size-related differences in hypoxic tolerance during the larva stage are also unlikely to result in adaptive, interpopulation variation in egg size. Although hypoxic tolerance is indeed lower for larvae from larger embryos, this effect is confined to the first half of the larval period when gills are underdeveloped and oxygen acquisition must be primarily cutaneous. Moreover, this effect of initial egg size on larval hypoxic tolerance is very small (Rombough 2007), so it is unlikely that hypoxic tolerance per se is responsible for the patterns observed in the present study.

On the other hand, metabolic rate increases relatively quickly with size in larvae, so a small larva requires less oxygen per unit of time than a larger larva, all else being equal (Rombough 2007). Assuming larval oxygen consumption scales at the rate of (embryo mass)$^{0.62}$ (Rombough 2007), we estimate that the per-capita oxygen consumption is roughly 16.7% higher in the sockeye population with the largest eggs compared with that with the smallest (Quinn et al. 1995). This difference may be important in small incubation gravels where flow rates are relatively low such that smaller larvae could persist in a given interstitial space for a longer period before running low on oxygen. We observed that total energy of juveniles increased with gravel size, which is indeed consistent with lower yolk-conversion efficiency in small-gravel environments (e.g., Olsson and Persson 1986) or at lower levels of dissolved oxygen per se (e.g., Mason 1969). We suggest that oxygen depletion rates by larvae, rather than hypoxic tolerance per se, may explain why large juveniles emerge prematurely from small gravels. In this scenario, however, larval biomass per unit area is likely an important factor that influences oxygen availability, but unfortunately, Quinn et al. (1995) do not report whether larval density (or fecundity) varies with gravel size among their focal populations.

If size-specific oxygen use by larvae is responsible for the patterns of emergence observed in the present study, then a novel hypothesis for the egg size – gravel size correlation arises. Although the relative importance of residual yolk and size-at-emergence on the probability of depredation is not understood, there is credible evidence to suggest that yolk-sac juveniles fare worse when depredation is high. For example, in a large-scale release programme spanning two years, juvenile survival was consistently and inversely related to a metric of residual yolk content, whereas selection on juvenile size was inconsistent and variable in direction (Fresh and Schroder 1987). An analysis of predator stomach contents in this study suggested that individuals with yolk sacs were being preferentially depredated (Fresh and Schroder 1987). Moreover, laboratory experiments have shown that yolk-sac larvae generally have a slower escape response than their fully developed counterparts (Hale 1996, 1999). If yolk-sac larvae are indeed more prone to depredation, then small individuals may have greater fitness when incubated in small gravels because they emerge as juveniles not as yolk-sac larvae. Although it is clear that yolk-sac juveniles also emerge under natural conditions (García De Leániz et al. 2000), our hypothesis depends critically on the benefits of increased size-at-emergence versus the propensity of these larger individuals with yolk sacs to be depredated, so further testing is certainly warranted.

Alternatively, the patterns observed in the present study may have nothing to do with the egg size – gravel size correlation observed by Quinn et al. (1995). First of all, it is not clear that a propensity to deplete local oxygen reserves in gravel interstices will necessarily result in premature emergence for larger larvae, as hypothesized above. Compaction stress in fine gravels, on the other hand, may encourage larvae of all sizes to emerge prematurely (Witzel and MacCrimmon 1983), but it may concomitantly result in delayed emergence in small larvae if small larvae are weaker and have more difficulty navigating the small interstitial spaces. This mechanism can explain every pattern that we observed in the present study, and this explanation is perhaps more parsimonious than the oxygen-stress hypothesis described above. It seems less likely that adaptive interpopulation egg-size variation would be maintained by size asymmetries in larval strength, as this explanation suggests that small juveniles expend more energy emerging from small gravels, which may have subsequent effects on growth and survival (e.g., Dias and Marshall 2010). Clearly, more research is needed to understand both the mechanism responsible for the pattern of emergence observed in the present study and to understand the egg size – gravel size correlation.

Another long-standing hypothesis for the egg size – gravel size correlation is that egg and embryo predators such as sculpins (*Cottus* sp.) and gobies (*Neogobius* sp.) are be better able to penetrate larger gravels, such that large eggs might be favoured in large gravels if predators are gape-limited (Quinn et al. 1995; Einum et al. 2002). We think that this hypothesis is unlikely. Although egg and embryo predators may be an important source of mortality (Foote and Brown 1998), there is little evidence that gobies and sculpins can differentially penetrate the gravel sizes described in the 21 populations of sockeye salmon (Biga et al. 1998; Chotkowski and Marsden 1999; Palm et al. 2009). Moreover, assuming that egg diameter (*d*; in mm) scales to egg wet mass according to the equation given by Einum and Fleming (2002),

$$(3) \qquad d = \left(6 \times v \times (1/\pi)\right)^{1/3}$$

where v is egg volume, and given that the range of egg masses described by Quinn et al. (1995) was 84.5–113.5 mg, this hypothesis requires that the predators be gape-limited to the extent that an estimated 0.56 mm difference in egg or embryo diameter decreases the likelihood of depredation in larger gravels.

A recent study also supports the finding that size-related survival of embryos and larvae may not occur in incubation gravels of different sizes (Rollinson and Hutchings 2011), though this study was limited in scope. The present study is a more thorough test of the focal hypothesis, but we note that it has three important drawbacks, any of which may explain why we observed no pattern of size-related survival. First, we cannot exclude the possibility that the original sockeye salmon populations (Quinn et al. 1995) exhibit traits at the embryo, larval, or maternal stage that act in concert with variation in egg size to effect the positive egg size – gravel size correlation. For example, the depth at which mothers bury their embryos may vary with spawning substrate (e.g., Kondolf and Wolman 1993), but we buried embryos at a depth of 23 cm at all levels of gravel size, which is the average burial depth of Atlantic salmon embryos (Devries 1997). Burial depth may correlate negatively with dissolved oxygen levels (e.g., Ingendahl 2001; Malcolm et al. 2004; but see Peterson and Quinn 1996) and given that large larvae have a higher per-capita oxygen consumption (Rombough 2007), mothers may do well to spawn small eggs in shallow nests when gravel size is small and flow is limited. We note that survival in our smallest gravel was very low at all levels of egg size, which underlines the possibility that the present study did not effectively emulate conditions in the wild. Second, the gravel sizes used in the present study were homogenous, and this is unlikely to occur in nature. A silt, sand, and cobble mixture, for example, may have the same geometric mean gravel size a homogeneous pebble mixture, but the two mixtures may not have the same effects on offspring survival or phenotypes (Tappel and Bjornn 1983). Our results may have differed if we had more closely emulated gravel mixtures found in the wild rather than using homogenous gravel mixtures. Third, and with the other caveats in mind, it is difficult to overlook the possibility that we committed a type II error with respect to size-related survival in different gravel size fractions. The relevant interaction term approached significance ($P = 0.07$), and small embryos appeared to fare similarly in both small and medium gravels, which would provide at least some support to the focal hypothesis.

In summary, our data do not support the hypothesis that size-related entombment of salmonid larvae occurs (Quinn et al. 1995; Quinn 2005), though caveats particular to the present experiment may have precluded a proper test of the focal hypothesis. We identify the possibility that interpopulation variation in egg size is maintained by size-related oxygen demand of larvae coupled with a propensity for larger larvae to deplete oxygen reserves more quickly in fine gravels, which ultimately results in premature emergence of large larvae from small gravels. It is also possible that large larvae emerge sooner from small gravel because they are simply stronger than larvae from small embryos, in which case it seems less likely that the pattern that we observed in the present study can explain the egg size – gravel size correlation. The present study shows that the mechanism(s) involved in effecting the positive correlation between egg size and gravel size, if any, may be more complex than originally anticipated.

Acknowledgements

The authors thank M. Yates, P. Debes, A.L.S. Houde, D. Keith, and J. Porter for laboratory and logistical assistance. We thank T. Quinn and an anonymous reviewer whose comprehensive reviews were invaluable in improving this manuscript. Research was supported by Fisheries and Oceans Canada, who provided lab space, gametes, and logistical assistance, and by a Natural Sciences and Engineering Research Council of Canada (NSERC) CGS grant to N. Rollinson and an NSERC discovery grant to J.A. Hutchings.

References

Allen, R.M., Buckley, Y.M., and Marshall, D.J. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. Am. Nat. **171**(2): 225–237. doi:10.1086/524952. PMID:18197775.

Bachman, R.A. 1984. Foraging behavior of free-ranging wild and hatchery reared brown trout in a stream. Trans. Am. Fish. Soc. **113**(1): 1–32. doi:10.1577/1548-8659(1984)113<1:FBOFWA>2.0.CO;2.

Bashey, F. 2006. Cross-generational environmental effects and the evolution of offspring size in the Trinidadian guppy *Poecilia reticulate*. Evolution, **60**(2): 348–361. PMID:16610325.

Berven, K.A., and Gill, D.E. 1983. Interpreting geographic variation in life-history traits. Am. Zool. **23**(1): 85–97.

Biga, H., Janssen, J., and Marsden, J.E. 1998. Effect of substrate size on lake trout egg predation by mottled sculpin. J. Great Lakes Res. **24**(2): 464–473. doi:10.1016/S0380-1330(98)70836-6.

Chotkowski, M.A., and Marsden, J.E. 1999. Round goby and mottled sculpin predation on lake trout eggs and fry: field predictions from laboratory experiments. J. Great Lakes Res. **25**(1): 26–35. doi:10.1016/S0380-1330(99)70714-8.

Congdon, J.D., and Gibbons, J.W. 1987. Morphological constraint on egg size: a challenge to optimal egg size theory? Proc. Natl. Acad. Sci. U.S.A. **84**(12): 4145–4147. doi:10.1073/pnas.84.12.4145. PMID:3473499.

DeVries, P. 1997. Riverine salmonid egg burial depths: review of published data and implications for scour studies. Can. J. Fish. Aquat. Sci. **54**(8): 1685–1698. doi:10.1139/cjfas-54-8-1685.

Dias, G.M., and Marshall, D.J. 2010. Does the relationship between offspring size and performance change across the life-history? Oikos, **119**(1): 154–162. doi:10.1111/j.1600-0706.2009.17725.x.

Dill, L.M., and Northcote, T.G. 1970. Effects of gravel size, egg depth and egg density on intragravel movement and emergence of coho salmon (*Oncorhynchus kisutch*) alevins. J. Fish. Res. Board Can. **27**(7): 1191–1199. doi:10.1139/f70-141.

Einum, S., and Fleming, I.A. 2000*a*. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). Evolution, **54**(2): 628–639. PMID:10937238.

Einum, S., and Fleming, I.A. 2000*b*. Highly fecund mothers sacrifice offspring survival to maximise fitness. Nature (London), **405**(6786): 565–567. doi:10.1038/35014600. PMID:10850714.

Einum, S., and Fleming, I.A. 2002. Does within-population variation in fish egg size reflect maternal influences on optimal values? Am. Nat. **160**(6): 756–765. doi:10.1086/343876. PMID:18707463.

Einum, S., Hendry, A.P., and Fleming, I.A. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proc. R. Soc. Lond. B Biol. Sci. **269**(1507): 2325–2330. doi:10.1098/rspb.2002.2150. PMID:12495499.

Einum, S., Kinnison, M.T., and Hendry, A.P. 2004. Evolution of egg size and number. *In* Evolution illuminated: salmon and their relatives. *Edited by* A.P. Hendry and S.C. Stearns. Oxford University Press, Oxford, UK. pp. 126–153.

Fischer, B., Taborsky, B., and Kokko, H. 2011. How to balance the offspring quality–quantity tradeoff when environmental cues are unreliable. Oikos, **120**(2): 258–270. doi:10.1111/j.1600-0706.2010.18642.x.

Foote, C.J., and Brown, G.S. 1998. Ecological relationship between freshwater sculpins (genus *Cottus*) and beach spawning sockeye salmon (*Oncorhynchus nerka*) in Iliamna Lake, Alaska. Can. J. Fish. Aquat. Sci. **55**(6): 1524–1533. doi:10.1139/f98-034.

Fox, C.W., Thakar, M.S., and Mousseau, T.A. 1997. Egg size plasticity in a seed beetle: an adaptive maternal effect. Am. Nat. **149**(1): 149–163. doi:10.1086/285983.

Fresh, K.L., and Schroder, S.L. 1987. Influence of the abundance, size and yolk reserves of juvenile chum salmon (*Oncorhynchus keta*) on predation by freshwater fishes in a small coastal stream. Can. J. Fish. Aquat. Sci. **44**(2): 236–243. doi:10.1139/f87-033.

García De Leániz, C., Fraser, N., and Huntingford, F.A. 2000. Variability in performance in wild Atlantic salmon, *Salmo salar* L., fry from a single redd. Fish. Manag. Ecol. **7**(6): 489–502. doi:10.1046/j.1365-2400.2000.00223.x.

Gregersen, F., Haugen, T.O., and Vøllestad, L.A. 2008. Contemporary egg size divergence among sympatric grayling demes with common ancestors. Ecol. Freshwat. Fish, **17**(1): 110–118. doi:10.1111/j.1600-0633.2007.00264.x.

Hale, M.E. 1996. The development of fast-start performance in fishes: escape kinematics of the Chinook salmon (*Oncorhynchus tshawytscha*). Am. Zool. **36**(6): 695–709.

Hale, M.E. 1999. Locomotor mechanics during early life history: effects of size and ontogeny on fast-start performance of salmonid fishes. J. Exp. Biol. **202**(11): 1465–1479. PMID:10229693.

Hendry, A.P., Day, T., and Cooper, A.B. 2001. Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. Am. Nat. **157**(4): 387–407. doi:10.1086/319316. PMID:18707249.

Houde, A.L.S., Fraser, D.J., O'Reilly, P., and Hutchings, J.A. 2011. Maternal and paternal effects on fitness correlates in outbred and inbred Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **68**(3): 534–549. doi:10.1139/F11-001.

Hutchings, J.A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. Evolution, **45**(5): 1162–1168. doi:10.2307/2409723.

Ingendahl, D. 2001. Dissolved oxygen concentration and emergence of sea trout fry from natural redds in tributaries of the River Rhine. J. Fish Biol. **58**(2): 325–341. doi:10.1111/j.1095-8649.2001.tb02256.x.

Kane, T.R. 1988. Relationship of temperature and time of initial feeding in Atlantic salmon. Prog. Fish-Cult. **50**(2): 93–97. doi:10.1577/1548-8640(1988)050<0093:ROTATO>2.3.CO;2.

Killeen, J., McLay, H.A., and Johnston, I.A. 1999. Development in *Salmo trutta* at different temperatures, with a quantitative scoring method for intraspecific comparisons. J. Fish Biol. **55**(2): 382–404. doi:10.1111/j.1095-8649.1999.tb00686.x.

Kondolf, G.M., and Wolman, M.G. 1993. The sizes of salmonid spawning gravels. Water Resour. Res. **29**(7): 2275–2285. doi:10.1029/93WR00402.

Krogh, A. 1959. The comparative physiology of respiratory mechanisms. University of Pennsylvania Press, Philadelphia, Pennsylvania.

Malcolm, I.A., Soulsby, C., Youngson, A.F., Hannah, D.M., McLaren, I.S., and Thorne, A. 2004. Hydrological influences on hyporheic water quality: implications for salmon egg survival. Hydrol. Process. **181**(9): 1543–1560.

Marshall, D.J., and Keough, M.J. 2008. The relationship between offspring size and performance in the sea. Am. Nat. **171**(2): 214–224. doi:10.1086/524954. PMID:18197774.

Mason, J.C. 1969. Hypoxial stress prior to emergence and competition among coho salmon fry. J. Fish. Res. Board Can. **26**(1): 63–91. doi:10.1139/f69-007.

Michaud, J., and Taggart, C.T. 2007. Lipid and gross energy content of North Atlantic right whale food, *Calanus finmarchicus*, in the Bay of Fundy. Endanger. Species Res. **3**: 77–94. doi:10.3354/esr003077.

Olsson, T.I., and Persson, B.G. 1986. Effects of gravel size and peat material concentrations on embryo survival and alevin emergence of brown trout, *Salmo trutta* L. Hydrobiologia, **135**(1–2): 9–14. doi:10.1007/BF00006453.

Palm, D., Lindberg, M., Brännäs, E., Lundqvist, H., Östergren, J., and Carlsson, U. 2009. Influence of European sculpin, *Cottus gobio*, on Atlantic salmon, *Salmo salar*, recruitment and the effect of gravel size on egg predation — implications for spawning habitat restoration. Fish. Manag. Ecol. **16**(6): 501–507. doi:10.1111/j.1365-2400.2009.00705.x.

Parciak, W. 2002. Seed size, number and habitat of a fleshy-fruited plant: consequences for seedling establishment. Ecology, **83**(3): 794–808. doi:10.1890/0012-9658(2002)083[0794:SSNAHO]2.0.CO;2.

Peterson, N.P., and Quinn, T.P. 1996. Spatial and temporal variation in dissolved oxygen in natural egg pockets of chum salmon, in Kennedy Creek, Washington. J. Fish Biol. **48**(1): 131–143. doi:10.1111/j.1095-8649.1996.tb01424.x.

Phillips, R.W., Lantz, R.L., Claire, E.W., and Moring, J.R. 1975. Some effects of gravel mixtures on emergence of coho salmon and steelhead trout fry. Trans. Am. Fish. Soc. **104**(3): 461–466. doi:10.1577/1548-8659(1975)104<461:SEOGMO>2.0.CO;2.

Quinn, T.P. 2005. The behaviour and ecology of Pacific salmon and trout. University of British Columbia Press, Vancouver, British Columbia.

Quinn, T.P., Hendry, A.P., and Wetzel, L.A. 1995. The influence of life history trade-offs and the size of incubation gravels on egg size variation on size variation in sockeye salmon (*Oncorhynchus nerka*). Oikos, **74**(3): 425–438. doi:10.2307/3545987.

Rollinson, N., and Hutchings, J.A. 2011. Body size-specific maternal effects on the offspring environment shape juvenile phenotypes in Atlantic salmon. Oecologia (Berl.). In press doi:10.1007/s00442-011-1945-9..

Rombough, P.J. 2007. Oxygen as a constraining factor in egg size evolution in salmonids. Can. J. Fish. Aquat. Sci. **64**(4): 692–699. doi:10.1139/f07-047.

Rose, H.E., and Rizk, A.M. 1949. Further researches in fluid flow through beds of granular material. Proc. Inst. Mech. Eng. **160**(1949): 493–511. doi:10.1243/PIME_PROC_1949_160_047_02.

Sakai, S., and Harada, Y. 2001. Why do large mothers produce large offspring? A theory and a test. Am. Nat. **157**(3): 348–359. doi:10.1086/319194. PMID:18707295.

Sinervo, B., and Licht, P. 1991. Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. Science (Washington, D.C.), **252**(5010): 1300–1302. doi:10.1126/science.252.5010.1300. PMID:17842955.

Smith, C.C., and Fretwell, S.D. 1974. The optimal balance between size and number of offspring. Am. Nat. **108**(962): 499–506. doi:10.1086/282929.

Tappel, P.D., and Bjornn, T.C. 1983. A new method of relating size of spawning gravel to salmonid embryo survival. N. Am. J. Fish. Manage. **3**(2): 123–135. doi:10.1577/1548-8659(1983)3<123:ANMORS>2.0.CO;2.

Witzel, L.D., and MacCrimmon, H.R. 1981. Role of gravel substrate on ova survival and alevin emergence of rainbow trout, *Salmo gairdneri*. Can. J. Zool. **59**(4): 629–636. doi:10.1139/z81-092.

Witzel, L.D., and MacCrimmon, H.R. 1983. Embryo survival and alevin emergence of brook charr, *Salvelinus fontinalis*, and brown trout, *Salmo trutta*, relative to redd gravel composition. Can. J. Zool. **61**(8): 1783–1792. doi:10.1139/z83-230.

Zar, J.H. 1984. Biostatistical analysis. 2nd ed. Prentice-Hall Inc., Englewood Cliffs, New Jersey.

Does timber harvest influence the dynamics of marine-derived nutrients in Southeast Alaska streams?

Peter S. Levi, Jennifer L. Tank, Scott D. Tiegs, Janine Rüegg, Dominic T. Chaloner, and Gary A. Lamberti

Abstract: Streams often rely on nutrient subsidies, and variation in nutrient delivery may alter the ecosystem response. Pacific salmon (*Oncorhynchus* spp.) provide marine-derived nutrients to their natal streams but also cause benthic disturbance, with the net effect determined by watershed and stream characteristics. To understand the factors contributing to variation in salmon-derived nutrients (SDN), we studied nutrient concentration and export in seven streams with varying physical characteristics due to timber harvest (e.g., channel complexity) over three years in Southeast Alaska, USA. Salmon increased concentrations and export of dissolved and particulate nitrogen, phosphorus, and carbon, but the magnitude of increase varied up to 41-fold among streams. The density of live salmon best predicted the increase in nutrient concentration and export, whereas the density of carcasses had a negligible effect. Nutrient export was predicted by transient storage before and after the salmon run. Streams in harvested watersheds with simplified channels had greater nutrient export than those in pristine watersheds with complex channels. However, enrichment from salmon overrode the effect of timber harvest on export during the run. Our study demonstrates that enrichment via SDN is short-lived and related to run size, whereas timber harvest and carcasses exert little influence on SDN dynamics.

Résumé : Les cours d'eau dépendent souvent de subsides de nutriments et la variation dans les apports de nutriments peut modifier les réactions de l'écosystème. Les saumons du Pacifique (*Oncorhynchus* spp.) procurent des nutriments d'origine marine à leur cours d'eau de naissance, mais ils causent aussi des bouleversements benthiques; l'effet net dépend des caractéristiques du bassin versant et du cours d'eau. Afin de connaître les facteurs qui contribuent à la variation dans les nutriments provenant des saumons (SDN), nous avons étudié les concentrations et les exportations de nutriments dans sept cours d'eau présentant des caractéristiques physiques différentes dues à la coupe du bois (par ex., complexité du chenal) pendant trois ans dans le sud-est de l'Alaska. Les saumons font augmenter les concentrations et l'exportation de l'azote dissous et particulaire, du phosphore et du carbone, mais l'importance de l'augmentation varie par un facteur pouvant atteindre 41 entre les cours d'eau. La densité des saumons est la meilleure variable prédictive de l'augmentation des concentrations et de l'exportation des nutriments, alors que la densité des carcasses a un effet négligeable. L'exportation des nutriments peut être prédite en tenant compte de l'accumulation temporaire avant et après la montaison des saumons. Les cours d'eau dans les bassins versants ayant subi la coupe forestière qui ont un chenal simplifié exportent plus de nutriments que ceux dans des bassins versants non affectés avec un chenal plus complexe. Cependant, l'enrichissement apporté par les saumons surpasse l'effet de la récolte du bois sur l'exportation durant la montaison. Notre étude démontre que l'enrichissement en SDN est de courte durée et qu'il est relié à la taille de la montaison, mais que le récolte du bois et les carcasses ont peu d'influence sur la dynamique des SDN.

[Traduit par la Rédaction]

Introduction

Stream ecosystems often depend on nutrient subsidies to maintain their structure and function (Polis et al. 1997). In coastal watersheds and tributaries to large lakes, the migration of anadromous fishes can be a major upstream vector of nutrients (e.g., Garman and Macko 1998; Walters et al. 2009). For example, Pacific salmon (*Oncorhynchus* spp.) deliver nitrogen (N), phosphorus (P), and carbon (C) to their natal streams and lakes throughout the Pacific Rim during their annual spawning migrations (Gende et al. 2002; Naiman et al. 2002). Previous research suggests that salmon-derived nutrients (SDN) are an important subsidy, particularly in low-nutrient stream ecosystems, as evidenced by increases in

Received 19 October 2010. Accepted 18 April 2011. Published at www.nrcresearchpress.com/cjfas on 28 July 2011.
J22075

Paper handled by Associate Editor John Richardson.

P.S. Levi, J.L. Tank, S.D. Tiegs,* J. Rüegg, D.T. Chaloner, and G.A. Lamberti. Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA.

Corresponding author: P.S. Levi (e-mail: plevi@nd.edu).

*Present address: Department of Biological Sciences, Oakland University, Rochester, Michigan, USA.

doi:10.1139/F2011-067

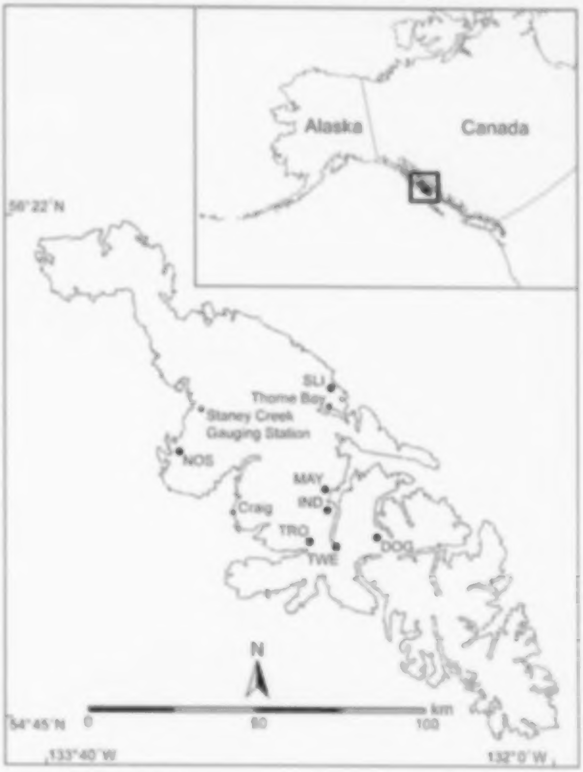
Published by NRC Research Press

primary producer biomass (e.g., Schindler et al. 2005), incorporation of SDN into higher trophic levels (e.g., Bilby et al. 1996), and alleviation of biofilm nutrient limitation (Rüegg et al. 2011). Although previous research confirms that salmon have the potential to enrich streams by elevating nutrient concentrations, recently, some have shown that salmon also disturb the benthos with their spawning activities (Moore et al. 2004; Tiegs et al. 2009). Salmon, therefore, have two potential effects in streams, namely as sources of enrichment and as agents of disturbance.

The net ecological effect of salmon in a stream may be influenced by watershed and stream characteristics such as land cover – land use, riparian vegetation, substrate composition, and channel complexity (Janetski et al. 2009; Holtgrieve et al. 2010). For example, streams with simplified channels dominated by fine sediments and low wood volume may undergo increased sediment and biofilm disturbance (Tiegs et al. 2008), potentially reducing SDN retention and increasing downstream nutrient export. In contrast, streams with higher geomorphic complexity and retention structures such as large sediments and high wood volume may retain more SDN and thus reduce nutrient export (Cederholm et al. 1989). Many watersheds with salmon-bearing streams in forests of the Pacific Northwest and Southeast Alaska have experienced some degree of timber harvest, which often simplifies stream channels (McIntosh et al. 2000) and may influence the net effect of salmon within a stream, be it predominantly enrichment or disturbance. Furthermore, the extent to which physical conditions affect variation in salmon enrichment or disturbance may depend on the specific form of the SDN.

Salmon deliver nutrients in different forms, including dissolved and particulate N, P, and C. These forms may indicate the degree of ecosystem enrichment or disturbance within a stream during the salmon run. Salmon provide highly labile forms of N and P as excretion of dissolved ammonium (NH_4^+) and soluble reactive phosphorus (SRP) from metabolic waste (Wood 1995) and the release of phospholipid-rich eggs and milt (Gende et al. 2002). Several studies also found an increase in nitrate (NO_3^-) concentrations during salmon runs (e.g., Johnston et al. 2004), although salmon do not directly excrete N as NO_3^- (Wood 1995). Salmon-derived NH_4^+ may enrich in-stream microbes, leading to increased rates of nitrogen transformations (e.g., nitrification), but not all salmon studies have observed such increases in nitrate (e.g., Chaloner et al. 2004, 2007; Hood et al. 2007). Furthermore, salmon may be a source of dissolved organic carbon to stream biota, thereby enriching primary producers and microbes (Hood et al. 2007; Rüegg et al. 2011). Increases in particulate N (PN), P (PP), and C (PC) during the salmon run may result from disturbance of benthic sediments or sloughing of dead salmon material (Moore et al. 2007; Tiegs et al. 2009). The form in which SDN are delivered to stream ecosystems will influence their biological uptake and export dynamics. For example, particulate nutrients will settle out from the water column more quickly than dissolved nutrients. Among dissolved nutrients, NO_3^-, being more difficult to assimilate than NH_4^+, may travel significantly farther downstream than other dissolved nutrients (Mulholland et al. 2000). Previous research has addressed the temporal and spatial variation in SDN to some extent, but no study has examined various nutrient forms and their interaction with physical factors such as watershed land use, stream structure, and discharge.

Fig. 1. Map of Prince of Wales Island in Southeast Alaska indicating our seven study streams (DOG, Dog Salmon; IND, Indian; MAY, Maybeso; NOS, Nossuk; SLI, Slide; TRO, Trocadero; TWE, Twelve-Mile) and the USGS Staney Creek gauging station.



The current literature demonstrates that Pacific salmon can enrich, as well as disturb, their natal streams during spawning runs; additionally, studies suggest that attributes of stream structure (e.g., sediment size) may influence their overall ecological role (Tiegs et al. 2008; Holtgrieve et al. 2010). However, rarely have studies quantified SDN dynamics at spatial and temporal scales greater than several streams in one year or one stream in several years (but see Moore et al. 2007). The objective of our research was to explain spatial and temporal variation in the magnitude of nutrient enrichment via SDN by studying multiple streams across multiple years. We quantified both dissolved and particulate nutrient concentrations and export in seven streams before, during, and after the salmon run for three years on Prince of Wales Island in Southeast Alaska. Our goal was to identify the physical variables that influence the stream nutrient response to salmon by sampling streams across a gradient of watershed timber harvest.

Materials and methods

Site description

Our research was conducted on Prince of Wales Island, Southeast Alaska, USA, during the summer and autumn of

Table 1. Physical characteristics of the seven study streams on Prince of Wales Island, Alaska. Streams are ordered in terms of increasing salmon run size from top to bottom.

Stream (abbreviation)	Average peak salmon density (salmon·m^{-2}) (±SE)	Watershed area (km^2)	Watershed harvested (%)	Baseflow discharge (L·s^{-1})	Baseflow width (m)	Large wood volume (m^3)	Sediment size (mm)	Storage area (A_S:A)
Trocadero (TRO)	0.05 (0.03)	44.8	11.2	477.8	13.6	72.5	88.7	0.26
Nossuk (NOS)	0.08 (0.004)	19.5	5.4	94.8	10.0	146.1	91.6	0.75
Slide (SLI)	0.11 (0.05)	26.0	57.9	278.7	9.8	21.5	118.4	0.43
Indian (IND)	0.46 (0.14)	25.9	9.3	272.6	8.8	178.0	75.9	0.83
Dog Salmon (DOG)	0.55 (0.21)	37.3	14.4	410.8	11.8	2.4	93.5	0.41
Maybeso (MAY)	0.56 (0.11)	38.7	21.8	608.1	18.5	17.9	46.5	0.13
Twelve-Mile (TWE)	0.58 (0.15)	31.1	68.3	519.1	12.3	11.8	32.5	0.20

Note: Average peak salmon density represents the average from 2006 to 2008. SE, standard error.

2006 to 2008 (Fig. 1). The majority of the island lies within the Tongass National Forest and consists of temperate rainforest where timber harvest has been a dominant land use. The majority of timber extraction on the island occurred between 1959 and 1986 (USDA Forest Service 1997). Forest management practices have left a heterogeneous landscape with varying areas of timber harvest and second-growth forest in each watershed, thereby providing an opportunity to evaluate timber-harvest effects in an area that is otherwise similar with regards to climate, soils, and geology.

We selected seven streams in similarly sized watersheds across a timber harvest gradient ranging from 5% to 70% of the total watershed harvested (Table 1). To quantify timber harvest and other watershed characteristics (e.g., total area, upstream river kilometres, riparian alder), we used geographic information system (GIS) data layers and ortho-photos provided by the USDA Forest Service. In each stream, we selected a representative 300 m study reach within 5 km of the estuary.

Salmon density and stream characteristics

Pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon are the dominant species of salmon that migrate to Prince of Wales Island, where they continue to spawn at historical densities (Quinn 2005). Runs occur during a six- to eight-week window in August and September. In our study streams, we estimated live salmon and carcass density using direct counts 4 to 16 times per stream per year, depending on the duration of the salmon run and site accessibility. We counted all salmon in a 4 m wide belt transect across the wetted width of the channel every 10 m, an area comprising 40% of our 300 m reach. We walked upstream along the riparian zone to minimize salmon disturbance in conducting the counts. To estimate salmon density, we scaled these counts to the reach and divided the total number of salmon by the wetted area of the channel. Although salmon were present above our study reaches, their densities within 1 km upstream were proportional to the density that we estimated in the reach (P.S. Levi, personal observation).

Stream water level was recorded every 30 min in seven streams in 2007 and in four streams in 2008 (IND, MAY, NOS, TWE) using Odyssey water capacitance meters (Dataflow Systems, Inc., Christchurch, New Zealand). We periodically measured stream water velocity at fixed transects using a Marsh-McBirney Flo-Mate flow meter (Hach Company, Loveland, Colorado, U.S.A.) to determine stream discharge (Gore 2006). To estimate continuous discharge over time, we created rating curves using corresponding discharge and water level measurements (r^2 = 0.79 to 0.99). We estimated daily mean discharge for all streams in 2006 and three streams in 2008 (DOG, SLI, TRO) using regressions (r^2 = 0.72 to 0.83) between daily mean discharge for each stream and the US Geological Survey gauging station on Staney Creek (no. 15081497; Fig. 1) in 2007.

To estimate mean sediment size, we used a gravelometer to measure sediments ranging in size from 2 to 180 mm and a metre stick for sediments greater than 180 mm (Bunte and Abt 2001). At every 10 m throughout each 300 m reach, we chose five random particles across the stream transect (n = 150). To quantify large wood volume, we applied the line-intersect method at every 10 m transect along the 300 m reach to measure the length and medial diameter of wood greater than 0.1 m in diameter and 2 m in length within the bank-full channel (Robison and Beschta 1990).

We used steady-state injections of rhodamine-WT (RWT) dye to estimate transient storage parameters, including the area of the storage zone (A_S) relative to area of the active channel (A) (Webster and Valett 2006). We injected RWT into a well-mixed portion of the stream (rate = 200 mL·min^{-1}; model RHB, Fluid Metering, Inc., Syosset, New York, U.S.A.) and measured concentrations every 30 s before, during, and after the injection using a calibrated Hydrolab Mini-Sonde (Hach Company) located 180 to 250 m downstream. We modeled the RWT breakthrough curve and used the advection–dispersion equation to estimate transient storage parameters (Hart 1995; Webster and Valett 2006).

Water chemistry sampling and analysis

We collected water samples from each stream on multiple dates before, during, and after the salmon run, ranging from 6 to 43 times per stream per year depending on the timing of the salmon run and site accessibility. In each stream in 2006, we sampled four transects at 100 m intervals but found no longitudinal differences in nutrient concentrations before, during, or after the salmon run and therefore modified our sampling regime for 2007 and 2008 to one downstream sampling point for each reach. During each sampling visit, we collected three replicate water samples and one to two particulate samples. We filtered the water through a Whatman GF/F (0.7 μm) into polyethylene bottles for analysis of dissolved ammonium (NH_4^+), soluble reactive phosphorus (SRP), and nitrate (NO_3^-) and saved the filters for PN, PP,

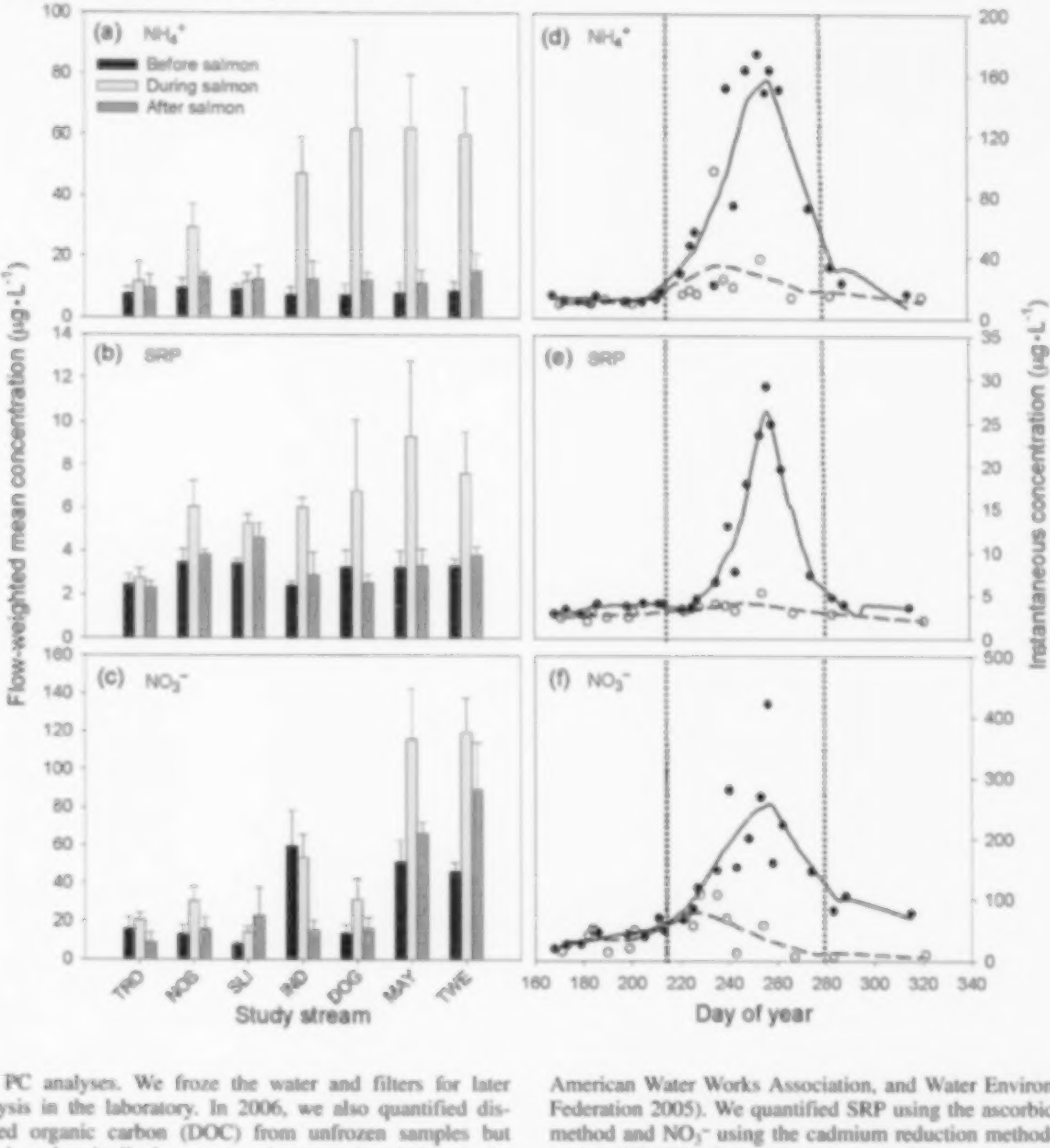


Fig. 2. Flow-weighted mean concentrations (+ standard error, SE) of dissolved (*a*) NH_4^+, (*b*) SRP, and (*c*) NO_3^- before, during, and after salmon from all years (2006–2008). Streams ($n = 7$) are arranged in order of increasing live salmon density based on the annual average at the peak of the run (see Table 1). Seasonal trends in dissolved (*d*) NH_4^+, (*e*) SRP, and (*f*) NO_3^- concentrations between streams with the largest and smallest salmon run size, with Twelve-Mile Creek (TWE) as solid circles (●) and Trocadero Creek (TRO) as open circles (○), in 2007. The trend lines indicate the running mean, calculated as the average of each value with the value immediately before and after each sampling date. The time period between broken lines indicates the salmon run. Streams: TRO, Trocadero; NOS, Nossuk; SLI, Slide; IND, Indian; DOG, Dog Salmon; MAY, Maybeso; TWE, Twelve-Mile.

and PC analyses. We froze the water and filters for later analysis in the laboratory. In 2006, we also quantified dissolved organic carbon (DOC) from unfrozen samples but found no longitudinal or temporal pattern in relation to the salmon run or watershed and stream characteristics. Therefore, we did not measure DOC in 2007 or 2008.

In the laboratory, we determined NH_4^+ concentrations using the phenol–hypochlorite method and a Shimadzu UV-1601 spectrophotometer (Shimadzu, Columbia, Maryland, USA; Solórzano 1969; American Public Health Association, American Water Works Association, and Water Environment Federation 2005). We quantified SRP using the ascorbic acid method and NO_3^- using the cadmium reduction method on a Lachat QC-8500 flow injection autoanalyzer (Lachat Instruments, Loveland, Colorado, USA; American Public Health Association, American Water Works Association, and Water Environment Federation 2005). We quantified PN and PC by combusting the filters on a Costech 4010 elemental analyzer (Costech Analytical, Valencia, California, U.S.A.). We determined PP using the same method as for SRP, but first di-

Table 2. Two-way RB-ANOVA statistical test results for flow-weighted mean concentrations of dissolved and particulate nutrients.

	Stream			Time period			Stream × time period			Year		
Nutrient	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
NH_4^+	6, 36	15.6	**<0.001**	2, 36	151.8	**<0.001**	12, 36	13.0	**<0.001**	2, 36	58.3	**<0.001**
SRP	6, 37	8.4	**<0.001**	2, 37	34.1	**<0.001**	12, 37	1.6	0.135	2, 37	11.0	**<0.001**
NO_3^-	6, 40	31.3	**<0.001**	2, 40	14.6	**<0.001**	12, 40	2.7	**0.010**	2, 40	10.9	**<0.001**
PN	6, 20	2.3	0.079	2, 20	6.1	**0.009**	12, 20	1.1	0.391	1, 20	0.3	0.566
PP	6, 13	12.4	**<0.001**	1, 13	181.9	**<0.001**	6, 13	5.3	**0.006**	1, 13	7.9	**0.015**
PC	6, 20	1.8	0.141	2, 20	7.2	**0.004**	12, 20	1.1	0.405	1, 20	3.2	0.088

Note: Bold *p* values indicate statistical significance ($p < 0.05$). NH_4^+, dissolved ammonium; SRP, soluble reactive phosphorus; NO_3^-, nitrate; PN, particulate nitrogen; PP, particulate phosphorus; PC, particulate carbon; df, degrees of freedom.

gested the sample in a hydrochloric acid solution (Stainton et al. 1977).

Because stream discharge varied through time, we calculated flow-weighted mean concentrations (FWMC) to normalize for differences in flow for all dissolved and particulate nutrients (Jordan et al. 1997). The FWMC was calculated for each seasonal time period (before, during, and after the salmon run) by dividing the total mass load of nutrient (sum of instantaneous concentration multiplied by the time step between samples) by the total stream discharge for that defined period. Our particulate nutrient sampling was too infrequent in 2006 to include all three years of data in the calculation of the FWMC. To calculate nutrient export (kg·day^{-1}), we divided the total mass load by the length, in days, of each seasonal time period.

Statistical analyses

To test for differences in nutrient dynamics among streams relative to the salmon run, we used a two-way randomized-block analysis of variance (RB-ANOVA, $\alpha = 0.05$; Zar 1999). The factors were stream and seasonal time period (i.e., before, during, and after the salmon run), with nutrient FWMC and export as the response variables, blocked by year. If ANOVA models were significant, we used a Tukey's post-hoc test for pair-wise comparisons of seasonal time periods or streams. When necessary, we transformed data to meet the assumptions of ANOVA. We could not normalize the particulate nutrient export data and used a Friedman nonparametric analysis to test the factors of seasonal time period and stream in lieu of the ANOVA (Zar 1999).

We used simple linear regression (SLR, $\alpha = 0.05$; Zar 1999) to determine the variables that best predicted nutrient concentration and export, including salmon density and transient storage (A_S:A). When necessary, we normalized data using log or square root transformations. We used analysis of covariance (ANCOVA, $\alpha = 0.05$; Zar 1999) to test for differences in nutrient legacy effects (e.g., hysteresis) within streams, with salmon density as the covariate. We performed all statistical analyses using SYSTAT 12 (Systat Software, Chicago, Illinois, U.S.A.).

Results

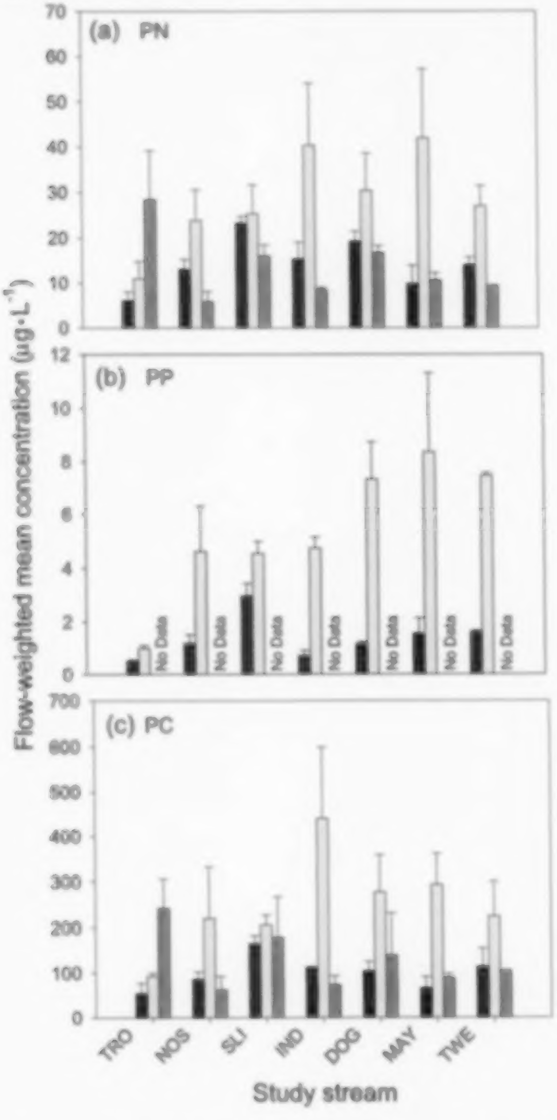
Nutrient concentration and export dynamics among streams and time periods

The presence of salmon increased the concentrations of all measured forms of dissolved nutrients. Before the salmon run, the FWMC of NH_4^+, SRP, and NO_3^- were low in all study streams and among all years (8.4, 3.1, and 29.7 μg·L^{-1}, respectively; Figs. 2*a*–2*c*). The background FWMC of NO_3^- was more variable among the streams compared with NH_4^+ and SRP (Appendix A, Table A1). During the salmon run, NH_4^+, SRP, and NO_3^- were higher, increasing as much as eightfold for NH_4^+ and threefold for both SRP and NO_3^- (Figs. 2*a*–2*c*). After the salmon run, NH_4^+ and SRP returned to before-salmon levels in all study streams; however, NO_3^- concentrations were variable and remained elevated in some streams while decreasing to before-salmon concentrations in others (Figs. 2*a*–2*c*). Differences in the FWMC of all nutrients varied among seasonal time periods (ANOVA, $p < 0.001$) and among streams (ANOVA, $p < 0.001$; Table 2). However, for NH_4^+ and NO_3^-, the response to the presence of salmon depended on the stream (significant interaction, $p \leq 0.01$). For SRP, the FWMC was significantly higher during the salmon run (Tukey's, $p < 0.001$) and also varied among streams, though did not result in clear groupings among streams.

Salmon increased particulate nutrient concentrations among all streams. Before the salmon run, FWMC of PN, PP, PC were variable among the seven streams (Appendix A, Table A1). During the salmon run, PN, PP, and PC increased significantly (ANOVA, $p \leq 0.009$; Figs. 3*a*–3*c*; Table 2). After the run, PN and PC decreased in six of the seven streams but remained variable. The temporal pattern in PN and PC did not vary among streams (ANOVA, $p > 0.07$; Table 2). However, PP did vary by stream (ANOVA, $p < 0.001$), but the variation was dependent on the seasonal time period (significant interaction, $p = 0.006$).

Salmon elevated stream water nutrient concentrations and, therefore, increased the downstream nutrient export from our study streams. Before the salmon run, daily NH_4^+ and SRP export were low and consistent among streams, whereas NO_3^- export was higher and more variable (0.5, 0.2, and 1.8 kg·day^{-1}, respectively; Appendix A, Table A2). During the run, the variation in NH_4^+ export ranged from 0.9 to 10.4 kg·day^{-1}, with NO_3^- export spanning a similar magnitude and range. Increased SRP export was generally an order of magnitude lower than that of dissolved nitrogen. After the salmon run, dissolved nutrient export remained elevated, especially NO_3^- in the heavily harvested watersheds (i.e., MAY, SLI, TWE). Dissolved NH_4^+ and NO_3^- export varied by the seasonal time period (ANOVA, $p < 0.05$) and by stream (ANOVA, $p < 0.001$) and was stream-specific (significant interaction, $p < 0.02$; Table 3). In contrast, SRP export did not vary by seasonal time period but did vary among streams (ANOVA, $p < 0.001$).

Fig. 3. Flow-weighted mean concentrations (+ standard error, SE) of particulate (*a*) nitrogen, (*b*) phosphorus, and (*c*) carbon before, during, and after the salmon run from two years (2007, 2008). Streams ($n = 7$) are arranged in order of increasing live salmon density at the peak of the run (see Table 1). Particulate phosphorus data were collected with low frequency after the salmon run and are thus not included in the figure. Streams: TRO, Trocadero; NOS, Nossuk; SLI, Slide; IND, Indian; DOG, Dog Salmon; MAY, Maybeso; TWE, Twelve-Mile.



In general, salmon increased daily export of particulate nutrients. Before the run, PP export was low, whereas PN and PC export were an order of magnitude higher (0.1, 1.1, and 8.0, respectively; Appendix A, Table A2). During the salmon run, particulate nutrient export increased in most streams, 1.5- to 18-fold, while remaining similar to before-salmon export for several nutrients in several streams. The export of particulate nutrients tended to be higher during the salmon run and in streams with large runs, but no significant differences were found among seasonal time periods (Friedman's test, $p \geq 0.3$) or among streams (Friedman's test, $p \geq 0.1$).

The seasonal pattern in dissolved nutrient concentrations closely tracked salmon density, as demonstrated in TWE and TRO, the streams with the highest and lowest live salmon density, respectively. NH_4^+ concentration consistently showed the greatest response to salmon (Figs. 2*d*–2*f*). Initially, NH_4^+ and NO_3^- increased synchronously with the arrival of salmon, whereas increases in SRP were delayed 7 to 10 days. The concentration of all dissolved nutrients peaked within 10 days of the recorded maximum salmon density and decreased with declines in salmon abundance. The particulate nutrients did not follow the unimodal pattern in salmon abundance.

Explaining the variation in stream nutrient response to the salmon subsidy

Changes in nutrient concentrations were largely explained by the density of live salmon. During the salmon run, NH_4^+, SRP, and NO_3^- were positively related to salmon density (Figs. 4*a*–4*c*). Furthermore, salmon density explained 28% to 30% of the variation in PN, PP, and PC (Figs. 4*d*–4*f*). Salmon density accounted for 48% of the variation in NH_4^+ compared with 24% to 30% in the other dissolved and particulate nutrients, suggesting that salmon exert the strongest influence on NH_4^+ concentrations, while the effect of salmon on the other dissolved and particulate nutrients is complicated by other factors. Thus, stream water nutrients were related to salmon density, but the degree to which nutrients increased in response to salmon varied among forms.

Despite the overall linear relationships between live salmon density and nutrients, dissolved nutrient concentrations within a stream and year demonstrated unique short-term legacy patterns. In some streams, nutrients increased and decreased linearly with live salmon density (e.g., MAY; ANCOVA interaction, $p = 0.9$, $F_{[1,11]} < 0.001$; Fig. 5*a*), whereas in other streams, nutrient concentrations remained elevated as live salmon densities decreased, creating a counterclockwise hysteresis pattern (e.g., TWE; ANCOVA significant interaction, $p = 0.02$, $F_{[1,12]} = 15.6$; Fig. 5*b*). Although the legacy patterns differed among streams, they were consistent within a stream among nutrients and years. For example, NH_4^+, SRP, and NO_3^- in Maybeso Creek increased and decreased linearly during salmon runs in all years, whereas these same nutrients showed hysteresis in TWE in all years. The hysteresis pattern was not observed for particulate nutrients.

Downstream nutrient export, including both the total mass of nutrients exported during the salmon run (kg) and daily average export (kg·day⁻¹), varied among the streams in relation to the size of the salmon run. The variation in total nutrient export (kg) during the salmon run among all streams and years was explained by the annual peak salmon density for all nutrients (SLR, R^2 range from 0.29 to 0.94; Figs. 6*a*–6*f*). Total nutrient export during the salmon run was not influenced by differences in stream structure across the timber harvest gradient.

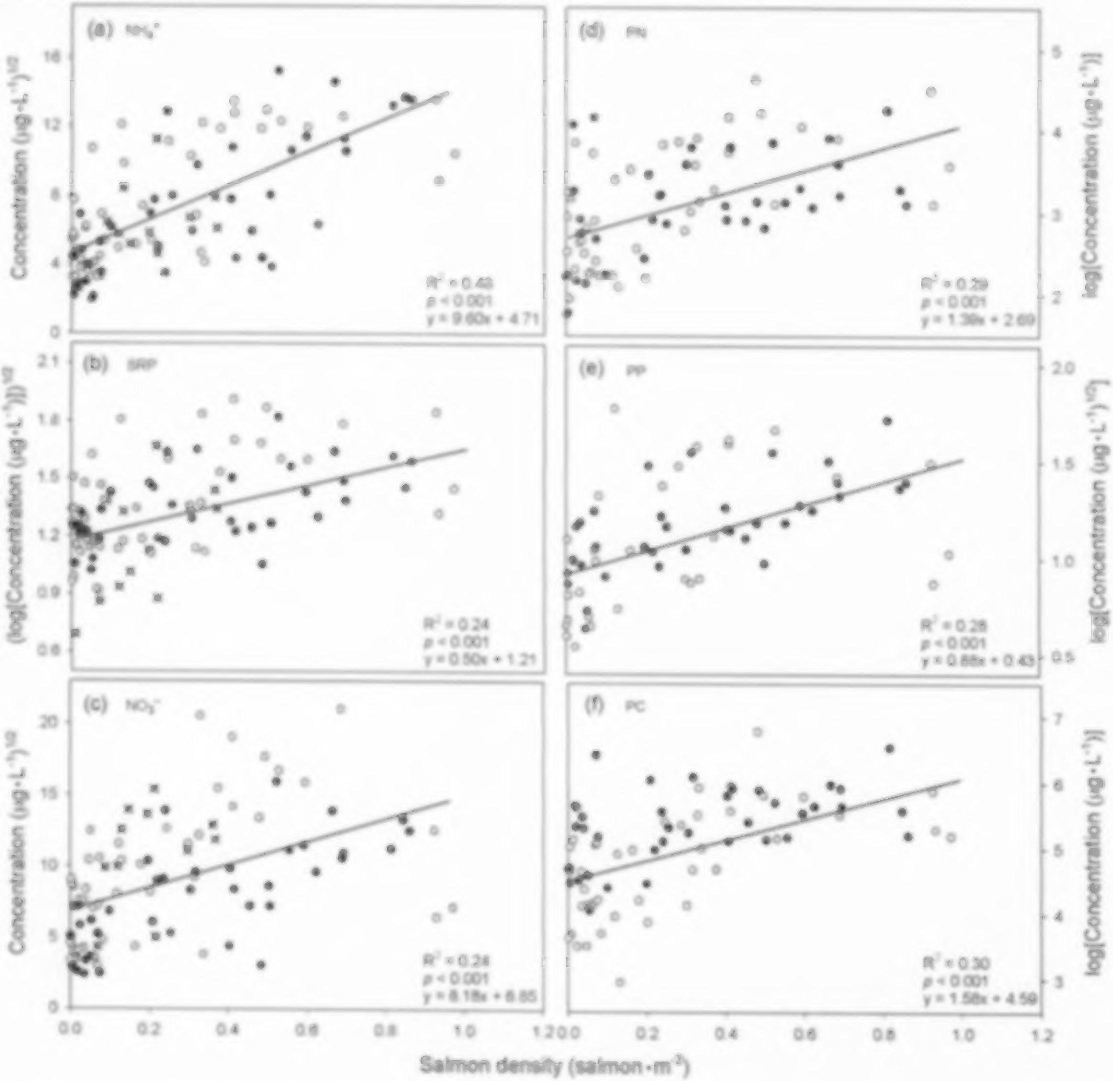
One of our predictions was that differences in channel complexity, as a result of watershed timber harvest, would influence the retention and export of SDN among streams and

Table 3. Two-way RB-ANOVA statistical test results for dissolved nutrient export.

	Stream			Time period			Stream × time period			Year		
Nutrient	df	F	p	df	F	p	df	F	p	df	F	p
NH_4^+	6, 32	10.1	**<0.001**	2, 32	24.6	**<0.001**	12, 32	3.1	**0.005**	2, 32	23.9	**<0.001**
SRP	6, 31	8.4	**<0.001**	2, 31	2.3	0.114	12, 31	1.1	0.435	2, 31	4.7	**0.016**
NO_3^-	6, 30	53.7	**<0.001**	2, 30	3.4	**0.048**	12, 30	2.7	**0.016**	2, 30	20.5	**<0.001**

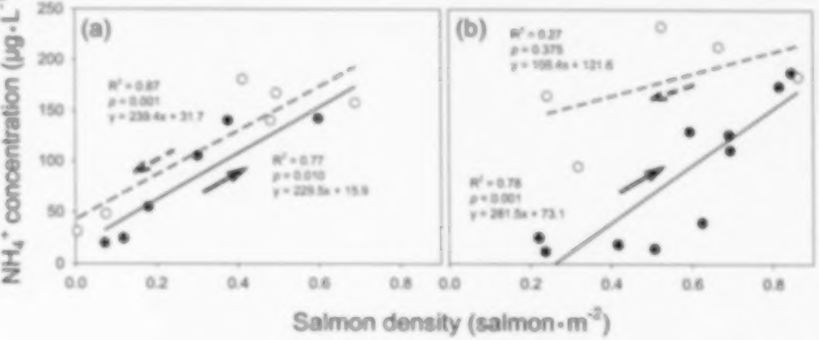
Note: Bold p values indicate statistical significance ($p < 0.05$). NH_4^+, dissolved ammonium; SRP, soluble reactive phosphorus; NO_3^-, nitrate; df, degrees of freedom.

Fig. 4. Linear regressions of live salmon density and dissolved (*a*) NH_4^+, (*b*) SRP, and (*c*) NO_3^- and particulate (*d*) nitrogen, (*e*) phosphorus, and (*f*) carbon concentrations across all streams ($n = 7$) and years (2006, ×; 2007, ○; 2008, ●). Particulate nutrient samples were not collected on days concurrent with salmon density counts in 2006. The data were normalized with square root (NH_4^+ and NO_3^-), square root log (SRP, PP), and log (PN, PC) transformations.



thus explain some variation in nutrient dynamics. Before the salmon run, export of ambient NH_4^+ and SRP was predicted by the relative size of the transient storage zone (A_S:A), an integrative metric of channel complexity (SLR: NH_4^+, $R^2 = 0.73$, $p = 0.01$; SRP, $R^2 = 0.84$, $p = 0.004$). However, during the salmon run, the relationship with A_S:A was no longer significant (SLR: NH_4^+, $R^2 = 0.31$, $p = 0.2$; SRP, $R^2 = 0.32$, $p = 0.2$). After the salmon run, export decreased slightly and

Fig. 5. Live salmon density and NH_4^+ concentration in (*a*) Maybeso Creek (2007) and (*b*) Twelve-Mile Creek (2008) demonstrating different patterns of the salmon-legacy effect. Solid circles (●) represent samples from the beginning of the run to peak salmon density (solid line), and open circles (○) represent samples from the peak salmon density to the end of the run (broken line). Solid and broken arrows indicate direction of nutrient increase and decrease, respectively, during the early and late periods of the salmon run.



the relationship with A_S:A was again significant for NH_4^+ (SLR, $R^2 = 0.66$, $p = 0.03$), but only marginally significant for SRP (SLR, $R^2 = 0.52$, $p = 0.07$). We observed no relationship in NO_3^- and particulate nutrient export with transient storage before, during, or after the salmon run.

Discussion

In this study, we quantified the delivery and export of dissolved and particulate salmon-derived nutrients to and from streams to determine potential environmental controls underlying the spatial and temporal variation of these subsidies. Consistent with previous studies, we found that salmon increased stream water dissolved and particulate nitrogen and phosphorus (see reviews by Gende et al. (2002) and Naiman et al. (2002)). However, nutrient concentrations were only elevated during the period when live salmon were present in the stream. Our results suggest that salmon carcasses had a negligible effect on stream water nutrients in contrast to other studies (Cederholm et al. 1999; Johnston et al. 2004). Furthermore, we found evidence that variation in stream characteristics due to previous watershed timber harvest, e.g., reduced channel complexity (McIntosh et al. 2000), mediated nutrient export when salmon were not present. However, enrichment by SDN during the salmon run overrode physical control of nutrient export. In general, live salmon increased nutrient export from spawning reaches to downstream habitats in proportion to their density.

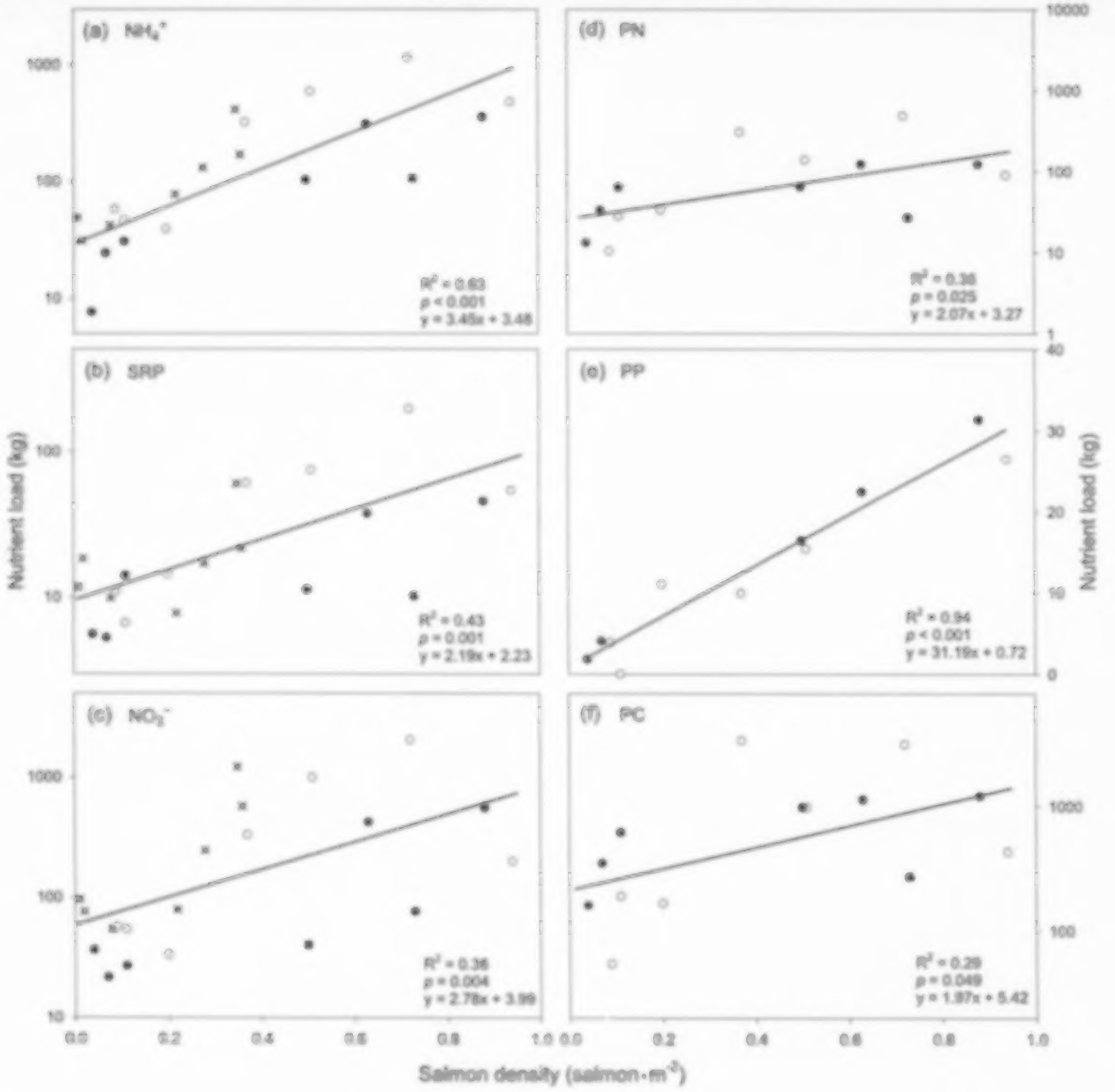
Influence of salmon on dissolved nutrient concentrations

The presence of live salmon increased dissolved N and P concentrations across multiple streams and years. During the salmon run, NH_4^+ and SRP increased dramatically above background concentrations, consistent with the majority of other salmon studies (Janetski et al. 2009). The magnitude with which NH_4^+ and SRP increased was variable among streams but was explained primarily by live salmon density, despite variability in environmental conditions across the study streams. As the salmon run declined, both NH_4^+ and SRP concentrations decreased despite increases in salmon carcass density, suggesting that the influence of carcasses on these dissolved nutrients was minimal. Because salmon excretion provides a source of NH_4^+ and SRP (Gende et al. 2002), it follows that dissolved nutrients would fluctuate in proportion to live salmon density. In our study, 48% of the variation in NH_4^+ among streams and years was explained by live salmon density (e.g., salmon excretion). However, salmon density accounted for less variation in stream water SRP, suggesting that salmon supply these nutrients to streams via different mechanisms (e.g., phosphorus-rich eggs and milt, bones). Water column concentrations of SRP are likely reflecting more than simply salmon contributions, including both abiotic and biotic variables in the sediments and water column.

Nitrate also increased dramatically during the salmon run despite salmon not directly excreting it (Wood 1995). Increases during the run could result from (*i*) in-stream microbial nitrification of salmon-derived NH_4^+ or (*ii*) flushing of terrestrial NO_3^- from riparian areas. In our study, discharge did not differ before or during the salmon run, but NO_3^- increased in all study streams and was predicted by live salmon density. Therefore, in-stream nitrification of salmon-derived NH_4^+ seems the most likely explanation for increases in NO_3^- during the salmon run. However, high discharge after the salmon run corresponded with elevated NO_3^- in the streams with the largest amount of watershed timber harvest (i.e., MAY, SLI, TWE). Red alder (*Alnus rubra*) is an N-fixing tree species common in disturbed areas, constituting up to 90% of the riparian area of streams in the heavily harvested watersheds (P.S. Levi, unpublished data), and is a significant contributor of NO_3^- to stream and riparian ecosystems (Compton et al. 2003). Therefore, increases in NO_3^- during the salmon run are likely due to in-stream nitrification of salmon-derived NH_4^+, whereas increases afterward are likely due to riparian soil flushing.

Salmon consistently increased NH_4^+, SRP, and NO_3^- concentrations in all streams, but temporal patterns were distinct among our study streams, suggesting differences in the short-term SDN legacy. These distinctive patterns demonstrate that, in addition to salmon density, stream and watershed characteristics may modify the influence of live salmon on dissolved nutrient dynamics. In three of our streams (IND, MAY, SLI), changes in SDN concentrations were directly related to changes in salmon density. However, in four of our

Fig. 6. Linear regressions of maximum live salmon density and total nutrient load (kg) exported from all streams ($n = 7$) of dissolved (*a*) NH_4^+, (*b*) SRP, and (*c*) NO_3^- during all years and particulate (*d*) nitrogen, (*e*) phosphorus, and (*f*) carbon for two years (2006, ×; 2007, ○; 2008, ●). All simple linear regressions were significant. Note that all *y* axes are on a log-scale except particulate phosphorus (*e*).



streams (DOG, NOS, TRO, TWE), nutrient concentrations remained elevated even as salmon numbers decreased, suggesting a short-term legacy effect (i.e., hysteresis) spanning several weeks after peak salmon density. The stream and watershed characteristics quantified in our study did not directly predict the patterns of hysteresis among streams or which streams would be likely to show such patterns. Previous studies have found longer-term storage of SDN on the scale of months to decades, for example, in the hyporheic zone (O'Keefe and Edwards 2003). However, we found consistently low NH_4^+ and SRP concentrations across all seven streams after the salmon run, despite a large range in run sizes among the streams, suggesting that the effect of salmon on stream water nutrients persisted only as long as live salmon were present.

Influence of salmon on particulate nutrient concentrations

Most SDN research has focused on the influence of salmon on dissolved nutrients (Janetski et al. (2009), but see Moore et al. (2007)). Our results demonstrate that salmon can directly increase particulate nutrient concentrations. Increases in PN, PP, and PC likely result from salmon-related benthic disturbance during migration and spawning (Moore et al. 2007) but may also result from sloughing of tissue from carcasses. Salmon exclusion studies have demonstrated that live salmon activity redistributes fine sediments and dislodges biota into the water column (Moore et al. 2004; Tiegs et al. 2009). Although our seven streams varied in mean sediment size, likely as a result of varying watershed timber harvest (Tiegs et al. 2008), the variation in particulate nutrients was still more strongly related to live salmon density than physical factors. Furthermore, we found no relationship between particulate nutrients and carcass density, suggesting that benthic disturbance by live salmon is the primary source of increased water column particulates rather than sloughed salmon tissue.

The increases in PN and PP were of smaller magnitude than the increases in the dissolved forms, suggesting that salmon have a more pronounced effect on dissolved N and P. These dissolved forms are excreted directly into the water column continuously, rather than as particulates primarily during spawning, and are more likely to remain suspended over time. Our results suggest that the effect of live salmon on dissolved and particulate nutrients is linear across a range of salmon densities, despite variation in watershed and stream characteristics.

Influence of salmon and watershed characteristics on downstream nutrient export

During the salmon run, the dissolved and particulate nutrient load exported from stream reaches was directly related to the size of the salmon run and, therefore, varied among the study streams, leading to a heterogeneous spatial pattern in downstream enrichment among watersheds. Dissolved and particulate N and P varied an order of magnitude among streams and years. The quantity and variability of nutrients exported from the study streams were similar to those previously reported for salmon-bearing streams in Southeast Alaska (Cak et al. 2008), although previous studies from this region did not relate the export directly to live salmon density. The thousands of anadromous salmon streams in Southeast Alaska could provide highly variable enrichment to their downstream estuaries simply based on the variation in salmon run size among streams. Estuaries are often considered N-limited (Howarth and Marino 2006), and large salmon runs in streams may temporarily alleviate the N limitation or shift nutrient limitation to phosphorus in downstream estuaries.

We initially hypothesized that simplified stream geomorphology, related to past timber harvest and reflected in low transient storage metrics (e.g., A_S:A), would influence the retention and export of SDN. Instead, channel complexity only appeared to influence NH_4^+ and SRP export during ambient conditions before and after the salmon run. During the salmon run, export of all nutrients was dominated by the SDN enrichment, which was proportional to salmon density. Previous studies have shown that the relative enrichment or disturbance effect of salmon can depend on stream and watershed characteristics (Tiegs et al. 2008; Holtgrieve et al. 2010). However, our results demonstrate that SDN export is not influenced by differences in transient storage but rather by the size of the salmon run.

Managing salmon to maintain SDN enrichment

Over the past century, the connectivity between migrating salmon and their natal spawning grounds has been disrupted over a significant portion of their historical range (Quinn 2005). Dams have limited access to upstream habitats, fishing pressure has reduced the magnitude of many salmon runs, and hatcheries have diverted salmon runs from natural streams (Stouder et al. 1997; Gresh et al. 2000). To simulate the annual pulse of nutrients to disconnected ecosystems, salmon carcasses and carcass analogues have been added to streams that no longer receive live runs (Wilzbach et al. 2005; Compton et al. 2006). Our results show that live salmon have the most pronounced effect on dissolved and particulate nutrients rather than natural carcasses, which demonstrates the importance of maintaining extant and restoring extinct runs. A recent meta-analysis comparing natural salmon runs and artificial salmon additions concluded that increases in dissolved nutrients were 5 to 10 times higher for natural runs than for carcass or analogue additions (Janetski et al. 2009). For instance, in a salmon carcass addition, Claeson et al. (2006) reported increases in NH_4^+ only, whereas for an analogue addition, Kohler et al. (2008) found no increases in any dissolved nutrients. Additionally, Wipfli et al. (2010) found that carcass additions increased NH_4^+ and SRP in stream mesocosms, but analogues only increased SRP. In our study, the carcass effect may have been minimal because the natural carcasses in our streams had lost a significant portion of their biomass and nutrients while alive, whereas fresh carcasses are typically used in artificial additions. Although artificial nutrient or carcass additions provide some temporary enrichment, it may be optimal to focus management efforts on restoring reduced or extinct runs because of the unique nature of the nutrient enrichment provided by live salmon.

Our study demonstrates that salmon run size influences the concentration and export of dissolved and particulate nutrients in streams. Live salmon more strongly influenced this relationship compared with their carcasses. In contrast, stream structure and watershed condition did not strongly influence stream water chemistry during the salmon run, but rather exerted control before and after the run. Restoration and conservation activities should ensure continued migrations of live salmon and their associated nutrient subsidies to their native streams, rivers, and lakes.

Acknowledgements

We are very grateful for the field and technical support provided by Mike Brueseke, Emily Campbell, Tyler Creech, Dave D'Amore, Rick Edwards, Susanne Hebbeler, Jim Junker, Ann Marie Larquier, Susan Meyer, Erik Norberg, Aaron Prussian, Katherine Prussian, Mia Stephen, Caroline Turner, and Sarah Winikoff. We also thank the Craig and Thorne Bay Ranger Districts for logistical support on Prince of Wales Island and the support of the Pacific Northwest Research Station (USDA Forest Service) Aquatic–Land Interactions Program. The research funding was provided by USDA-CSREES National Research Initiative (Managed Ecosystems Program 2006-35101-16566).

References

American Public Health Association, American Water Works Association, and Water Environment Federation. 2005. Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, Washington, D.C.

Bilby, R.E., Fransen, B.R., and Bisson, P.A. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. Can. J. Fish. Aquat. Sci. **53**(1): 164–173. doi:10.1139/f95-159.

Bunte, K., and Abt, S.R. 2001. Sampling frame for improving pebble count accuracy in coarse gravel-bed streams. J. Am. Water Resour. Assoc. **37**(4): 1001–1014. doi:10.1111/j.1752-1688.2001.tb05528.x.

Cak, A.D., Chaloner, D.T., and Lamberti, G.A. 2008. Effects of spawning salmon on dissolved nutrients and epilithon in coupled stream–estuary systems of southeastern Alaska. Aquat. Sci. **70**(2): 169–178. doi:10.1007/s00027-008-8090-5.

Cederholm, C.J., Houston, D.B., Cole, D.L., and Scarlett, W.J. 1989. Fate of coho salmon (*Oncorhynchus kisutch*) carcasses in spawning streams. Can. J. Fish. Aquat. Sci. **46**(8): 1347–1355. doi:10.1139/f89-173.

Cederholm, C.J., Kunze, M.D., Murota, T., and Sibatani, A. 1999. Pacific salmon carcasses: essential contributions of nutrients and energy for aquatic and terrestrial ecosystems. Fisheries (Bethesda), **24**(10): 6–15.

Chaloner, D.T., Lamberti, G.A., Merritt, R.W., Mitchell, N.L., Ostrom, P.H., and Wipfli, M.S. 2004. Variation in responses to spawning Pacific salmon among three south-eastern Alaska streams. Freshw. Biol. **49**(5): 587–599. doi:10.1111/j.1365-2427.2004.01213.x.

Chaloner, D.T., Lamberti, G.A., Cak, A.D., Blair, N.L., and Edwards, R.T. 2007. Inter-annual variation in responses of water chemistry and epilithon to Pacific salmon spawners in an Alaskan stream. Freshw. Biol. **52**(3): 478–490. doi:10.1111/j.1365-2427.2006.01715.x.

Claeson, S.M., Li, J.L., Compton, J.E., and Bisson, P.A. 2006. Response of nutrients, biofilm, and benthic insects to salmon carcass addition. Can. J. Fish. Aquat. Sci. **63**(6): 1230–1241. doi:10.1139/f06-029.

Compton, J.E., Church, M.R., Larned, S.T., and Hogsett, W.E. 2003. Nitrogen export from forested watersheds in the Oregon Coast Range: the role of N_2-fixing red alder. Ecosystems (N.Y.), **6**(8): 773–785. doi:10.1007/s10021-002-0207-4.

Compton, J.E., Andersen, C.P., Phillips, D.L., Brooks, J.R., Johnson, M.G., Church, M.R., Hogsett, W.E., Cairns, M.A., Rygiewicz, P. T., McComb, B.C., and Shaff, C.D. 2006. Ecological and water quality consequences of nutrient addition for salmon restoration in the Pacific Northwest. Front. Ecol. Environ, **4**(1): 18–26. doi:10.1890/1540-9295(2006)004[0018:EAWQCO]2.0.CO;2.

Garman, G.C., and Macko, S.A. 1998. Contribution of marine-derived organic matter to an Atlantic coast, freshwater, tidal stream by anadromous clupeid fishes. J. N. Am. Benthol. Soc. **17**(3): 277–285. doi:10.2307/1468331.

Gende, S.M., Edwards, R.T., Willson, M.F., and Wipfli, M.S. 2002. Pacific salmon in aquatic and terrestrial ecosystems. Bioscience, **52**(10): 917–928. doi:10.1641/0006-3568(2002)052[0917:PSIAAT]2.0.CO;2.

Gore, J.A. 2006. Discharge measurements and streamflow analysis. *In* Methods in stream ecology. 2nd ed. *Edited by* F.R. Hauer and G.A. Lamberti. Academic Press, San Diego, California. pp. 51–77

Gresh, T., Lichatowich, J., and Schoonmaker, P. 2000. An estimation of historic and current levels of salmon production in the Northeast Pacific ecosystem: evidence of a nutrient deficit in the freshwater systems of the Pacific Northwest. Fisheries (Bethesda), **25**(1): 15–21.

Hart, D.R. 1995. Parameter-estimation and stochastic interpretation of the transient storage model for solute transport in streams. Water Resour. Res. **31**(2): 323–328. doi:10.1029/94WR02739.

Holtgrieve, G.W., Schindler, D.E., Gowell, C.P., Ruff, C.P., and Lisi, P.J. 2010. Stream geomorphology regulates the effects on periphyton of ecosystem engineering and nutrient enrichment by Pacific salmon. Freshw. Biol. **55**(12): 2598–2611. doi:10.1111/j.1365-2427.2010.02489.x.

Hood, E., Fellman, J., and Edwards, R.T. 2007. Salmon influences on dissolved organic matter in a coastal temperate brown-water stream: an application of fluorescence spectroscopy. Limnol. Oceanogr. **51**(4): 364–376.

Howarth, R.W., and Marino, R. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades. Limnol. Oceanogr. **51**(1, part 2): 364–376. doi:10.4319/lo.2006.51.1_part_2.0364.

Janetski, D.J., Chaloner, D.T., Tiegs, S.D., and Lamberti, G.A. 2009. Pacific salmon effects on stream ecosystems: a quantitative synthesis. Oecologia, **159**(3): 583–595. doi:10.1007/s00442-008-1249-x. PMID:19132408.

Johnston, N.T., MacIsaac, E.A., Tschaplinski, P.J., and Hall, K.J. 2004. Effects of the abundance of spawning sockeye salmon (*Oncorhynchus nerka*) on nutrients and algal biomass in forested streams. Can. J. Fish. Aquat. Sci. **61**(3): 384–403. doi:10.1139/f03-172.

Jordan, T.E., Correll, D.L., and Weller, D.E. 1997. Relating nutrient discharges from watersheds to land use and streamflow variability. Water Resour. Res. **33**(11): 2579–2590. doi:10.1029/97WR02005.

Kohler, A.E., Rugenski, A., and Taki, D. 2008. Stream food web response to a salmon carcass analogue addition in two central Idaho, USA, streams. Freshw. Biol. **53**(3): 446–460. doi:10.1111/j.1365-2427.2007.01909.x.

McIntosh, B.A., Sedell, J.R., Thurow, R.F., Clarke, S.E., and Chandler, G.L. 2000. Historical changes in pool habitats in the Columbia River Basin. Ecol. Appl. **10**(5): 1478–1496. doi:10.1890/1051-0761(2000)010[1478:HCIPHI]2.0.CO;2.

Moore, J.W., Schindler, D.E., and Scheuerell, M.D. 2004. Disturbance of freshwater habitats by anadromous salmon in Alaska. Oecologia, **139**(2): 298–308. doi:10.1007/s00442-004-1509-3. PMID:14997375.

Moore, J.W., Schindler, D.E., Carter, J.L., Fox, J., Griffiths, J., and Holtgrieve, G.W. 2007. Biotic control of stream fluxes: spawning salmon drive nutrient and matter export. Ecology, **88**(5): 1278–1291. doi:10.1890/06-0782. PMID:17536413.

Mulholland, P.J., Tank, J.L., Sanzone, D.M., Wollheim, W.M., Peterson, B.J., Webster, J.R., and Meyer, J.L. 2000. Nitrogen cycling in a forest stream determined by a N-15 tracer addition. Ecol. Monogr. **70**(3): 471–493.

Naiman, R.J., Bilby, R.E., Schindler, D.E., and Helfield, J.M. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems (N.Y.), **5**(4): 399–417. doi:10.1007/s10021-001-0083-3.

O'Keefe, T.C., and Edwards, R.T. 2003. Evidence for hyporheic transfer and removal of marine-derived nutrients in a Sockeye stream in Southwest Alaska. *In* Nutrients in salmonid ecosystems: sustaining production and biodiversity. *Edited by* J. Stockner. American Fisheries Society, Herndon, Virginia. pp. 99–107.

Polis, G.A., Anderson, W.B., and Holt, R.D. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu. Rev. Ecol. Syst. **28**(1): 289–316. doi:10.1146/annurev.ecolsys.28.1.289.

Quinn, T.P. 2005. The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle, Washington.

Robison, E.G., and Beschta, R.L. 1990. Characteristics of coarse woody debris for several coastal streams of Southeast Alaska, USA. Can. J. Fish. Aquat. Sci. **47**(9): 1684–1693. doi:10.1139/f90-193.

Rüegg, J., Tiegs, S.D., Chaloner, D.T., Levi, P.S., Tank, J.L., and Lamberti, G.A. 2011. Salmon subsidies alleviate nutrient limitation of benthic biofilms in Southeast Alaska streams. Can. J. Fish. Aquat. Sci. **68**(2): 277–287. doi:10.1139/F10-145.

Schindler, D.E., Leavitt, P.R., Brock, C.S., Johnson, S.P., and Quay, P.D. 2005. Marine-derived nutrients, commercial fisheries, and production of salmon and lake algae in Alaska. Ecology, **86**(12): 3225–3231. doi:10.1890/04-1730.

Solórzano, L. 1969. Determination of ammonia in natural waters by phenolhypochlorite method. Limnol. Oceanogr. **14**(5): 799–801. doi:10.4319/lo.1969.14.5.0799.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of fresh-water. Freshwater Institute, Winnipeg, Manitoba, Misc. Spec. Publ. No. 25.

Stouder, D.J., Bisson, P.A., and Naiman, R.J. (*Editors*). 1997. Pacific salmon and their ecosystems: status and future options. Chapman and Hall, New York.

Tiegs, S.D., Chaloner, D.T., Levi, P., Rüegg, J., Tank, J.L., and Lamberti, G.A. 2008. Timber harvest transforms ecological roles of salmon in Southeast Alaska rain forest streams. Ecol. Appl. **18**(1): 4–11. doi:10.1890/07-0655.1. PMID:18372551.

Tiegs, S.D., Campbell, E.Y., Levi, P.S., Rüegg, J., Benbow, M.E., Chaloner, D.T., Merritt, R.W., Tank, J.L., and Lamberti, G.A. 2009. Separating physical disturbance and nutrient enrichment caused by Pacific salmon in stream ecosystems. Freshw. Biol. **54**(9): 1864–1875. doi:10.1111/j.1365-2427.2009.02232.x.

USDA Forest Service. 1997. Tongass land management plan revision. Final Environmental Impact Statement, Region 10 (Alaska Region). U.S. Department of Agriculture Forest Service, Juneau, Alaska.

Walters, A.W., Barnes, R.T., and Post, D.M. 2009. Anadromous alewives (*Alosa pseudoharengus*) contribute marine-derived nutrients to coastal stream food webs. Can. J. Fish. Aquat. Sci. **66**(3): 439–448. doi:10.1139/F09-008.

Webster, J.R., and Valett, H.M. 2006. Solute dynamics. *In* Methods in stream ecology. 2nd ed. *Edited by* F.R. Hauer and G.A. Lamberti. Academic Press, San Diego, California. pp. 169–186.

Wilzbach, M.A., Harvey, B.C., White, J.L., and Nakamoto, R.J. 2005. Effects of riparian canopy opening and salmon carcass addition on the abundance and growth of resident salmonids. Can. J. Fish. Aquat. Sci. **62**(1): 58–67. doi:10.1139/f04-177.

Wipfli, M.S., Hudson, J.P., Caouette, J.P., Mitchell, N.L., Lessard, J.L., Heintz, R.A., and Chaloner, D.T. 2010. Salmon carcasses increase stream productivity more than inorganic fertilizer pellets: a test on multiple trophic levels in streamside experimental channels. Trans. Am. Fish. Soc. **139**(3): 824–839. doi:10.1577/T09-114.1.

Wood, C.M. 1995. Excretion. *In* Physiological ecology of Pacific salmon. *Edited by* C. Groot, L. Margolis, and W.C. Clarke. UBC Press, Vancouver, British Columbia. pp. 381–438.

Zar, J.H. 1999. Biostatistical analysis. 4th ed. Prentice-Hall, Upper Saddle River, New Jersey.

Appendix A

Tables A1 and A2 follow.

Table A1. Flow-weighted mean nutrient concentrations (µg·L^{-1}) from all streams (n = 7) across all years (n = 3). Streams are ordered in
Dash (—) indicates that no data are available; particulate nutrients were not collected for 2006, and particulate phosphorus data were not

		Ammonium (NH_4^+-N)			Nitrate (NO_3^--N)			Phosphorus (SRP)			Particulate nitrogen (PN)		
	Year	Before	During	After	Before	During	After	Before	During	After	Before	During	After
TRO	2006	9.2	7.8	13.2	10.6	15.2	18.5	1.7	1.9	1.9	—	—	—
	2007	10.8	23.7	14.7	28.3	27.6	6.9	2.6	3.4	2.9	3.7	14.8	28.7
	2008	3.0	4.1	1.8	9.3	19.6	1.7	3.1	3.0	2.0	7.5	7.1	7.3
NOS	2006	11.7	22.2	13.0	4.3	28.7	28.0	2.5	5.2	3.5	—	—	—
	2007	13.7	45.0	15.6	15.7	44.2	9.1	3.4	8.4	4.3	13.8	15.4	4.5
	2008	4.0	21.2	10.8	20.1	18.8	11.1	4.6	4.6	3.7	9.5	28.8	9.0
SLI	2006	9.7	8.1	13.2	7.8	19.9	52.0	3.1	4.8	4.0	—	—	—
	2007	11.9	16.7	19.8	10.2	14.2	12.0	3.6	6.1	5.9	25.4	34.8	13.9
	2008	6.3	10.8	4.8	5.6	9.5	4.5	3.7	4.9	3.9	22.5	22.2	18.8
IND	2006	9.4	40.2	12.4	76.4	75.8	18.6	2.2	5.2	2.2	—	—	—
	2007	10.7	32.3	22.7	80.9	33.3	22.0	2.3	6.1	4.9	20.5	45.3	8.6
	2008	2.2	70.4	2.6	21.4	51.1	4.8	2.8	6.7	1.6	13.1	17.8	7.6
DOG	2006	8.9	29.0	12.8	22.8	29.6	25.5	4.8	2.9	1.8	—	—	—
	2007	12.6	120	16.4	9.1	50.3	16.4	2.2	13.4	3.1	20.5	39.3	16.5
	2008	1.2	36.8	7.4	9.7	14.4	7.6	2.8	4.0	2.7	16.0	23.0	19.6
MAY	2006	11.0	34.2	12.6	73.8	101	77.8	2.6	5.0	2.4	—	—	—
	2007	12.2	94.8	18.0	39.8	168	62.5	2.5	16.1	4.9	12.0	53.4	10.6
	2008	1.4	57.4	3.3	39.2	79.1	59.4	4.7	6.9	2.6	3.9	22.8	7.3
TWE	2006	10.3	36.1	18.8	51.8	122	123	2.7	4.6	4.5	—	—	—
	2007	13.1	89.2	23.0	37.0	150	105	3.5	11.2	3.9	11.6	23.6	9.4
	2008	3.5	55.2	3.7	49.3	86.4	41.5	3.7	7.0	3.1	15.0	32.3	—

Note: Streams: TRO, Trocadero; NOS, Nossuk; SLI, Slide; IND, Indian; DOG, Dog Salmon; MAY, Maybeso; TWE, Twelve-Mile.

Table A2. Nutrient flux (kg·day^{-1}) before, during, and after the salmon run calculated from flow-weighted nutrient concentrations from all
changes associated with the salmon run, with a ratio >1 indicating an increase in nutrient flux during the salmon run. Streams are ordered in

	Ammonium (NH_4^+-N)				Phosphorus (SRP)				Nitrate (NO_3^--N)			
Stream	Before	During	After	Ratio	Before	During	After	Ratio	Before	During	After	Ratio
TRO	0.6	1.0	1.9	1.6	0.3	0.2	0.4	0.7	1.6	1.7	1.3	1.1
NOS	0.1	1.2	0.8	9.2	0.04	0.3	0.2	5.9	0.2	1.3	1.1	7.3
SLI	0.7	0.9	1.6	1.3	0.3	0.4	0.5	1.5	0.6	1.2	3.5	2.0
IND	0.2	3.2	1.1	15.7	0.1	0.5	0.2	6.0	1.6	4.1	1.4	2.5
DOG	0.4	3.7	1.1	8.8	0.2	0.4	0.2	2.5	0.6	2.1	1.4	3.4
MAY	1.0	10.4	4.0	10.3	0.4	1.6	1.0	3.9	4.9	21.5	18.7	4.4
TWE	0.5	6.4	2.8	12.4	0.3	0.8	0.6	3.1	3.3	12.7	14.6	3.9

Note: Streams: TRO, Trocadero; NOS, Nossuk; SLI, Slide; IND, Indian; DOG, Dog Salmon; MAY, Maybeso; TWE, Twelve-Mile.

terms of increasing salmon run size from top to bottom.
collected after salmon in all years.

Particulate phosphorus (PP)			Particulate carbon (PC)		
Before	During	After	Before	During	After
—	—	—	—	—	—
0.5	1.2	—	24.7	100	244
0.5	1.0	—	71.1	85.7	117
—	—	—	—	—	—
1.3	6.9	—	91.9	80.3	43.3
0.6	3.5	—	59.6	303	105
—	—	—	—	—	—
3.4	5.1	—	191	174	102
2.5	4.2	—	157	218	279
—	—	—	—	—	—
0.4	5.1	—	112	498	69.8
0.7	4.3	—	113	180	110
—	—	—	—	—	—
1.2	8.7	—	92.6	189	128
1.1	5.9	—	135	352	314
—	—	—	—	—	—
1.7	10.0	—	79.9	344	89.0
0.6	4.1	—	28.2	210	108
—	—	—	—	—	—
1.5	7.3	—	55.2	166	106
1.6	7.5	—	137	317	—

streams ($n = 7$) across all years ($n = 3$). Ratio (during salmon to before salmon) indicates
terms of increasing salmon run size from top to bottom.

Particulate nitrogen			Particulate phosphorus			Particulate carbon		
Before	During	Ratio	Before	During	Ratio	Before	During	Ratio
0.8	0.6	0.8	0.03	0.02	0.7	7.2	5.0	0.7
0.2	0.6	3.0	0.02	0.1	5.3	1.2	5.1	4.3
1.7	1.3	0.8	0.3	0.3	1.0	11.8	10.9	0.9
0.8	2.1	2.7	0.01	0.1	18.1	5.3	23.2	4.4
1.6	1.2	0.7	0.1	0.3	3.6	10.5	11.2	1.1
1.4	4.5	3.3	0.3	1.2	4.6	9.2	31.7	3.5
1.4	2.2	1.6	0.2	0.4	3.0	11.1	19.2	1.7

A new method to reconstruct fish diet and movement patterns from $\delta^{13}C$ values in otolith amino acids

Kelton W. McMahon, Marilyn L. Fogel, Beverly J. Johnson, Leah A. Houghton, and Simon R. Thorrold

Abstract: Fish ecologists have used geochemical values in otoliths to examine habitat use, migration, and population connectivity for decades. However, it remains difficult to determine an unambiguous dietary $\delta^{13}C$ signature from bulk analysis of otolith. Studies to date have focused on the aragonite component of otoliths with less attention paid to the organic fraction. We describe the application of compound-specific stable isotope analysis (SIA) to analyze amino acid (AA) $\delta^{13}C$ values from small amounts (<1 mg) of otolith powder. We examined $\delta^{13}C$ values of otolith and muscle AAs from a reef-associated snapper (*Lutjanus ehrenbergii* (Peters, 1869)) collected along a carbon isotope gradient (isoscape) from seagrass beds to coral reefs. Carbon isotope values in otolith and muscle samples were highly correlated within and among coastal habitats. Moreover, $\delta^{13}C$ values of otolith AAs provided a purely dietary record that avoided dilution from dissolved inorganic carbon. Otolith AAs served as a robust tracer of $\delta^{13}C$ values at the base of the food web, making compound-specific SIA a powerful tool for dietary reconstructions and tracking the movement of fishes across isoscapes.

Résumé : Depuis des décennies, les écologistes des poissons emploient les valeurs géochimiques des otolithes pour étudier l'utilisation de l'habitat, la migration et la connectivité entre les populations. Il reste, cependant, difficile de déterminer une signature $\delta^{13}C$ alimentaire non ambiguë par analyse globale de l'otolithe. Jusqu'à maintenant, les études se sont intéressées à la composante aragonite des otolithes et ont porté moins d'attention à la fraction organique. Nous décrivons l'utilisation de l'analyse des isotopes stables (SIA) de composés spécifiques pour l'analyse des valeurs de $\delta^{13}C$ des acides aminés (AA) sur de petites quantités (<1 mg) de poudre d'otolithe. Nous avons déterminé les valeurs de $\delta^{13}C$ des AA du muscle et des otolithes chez des vivaneaux (*Lutjanus ehrenbergii* (Peters, 1869)) associés aux récifs et prélevés le long d'un gradient d'isotopes de carbone (isoscape) allant d'herbiers marins à des récifs coralliens. Les valeurs des isotopes de carbone dans les otolithes et les échantillons de muscle sont fortement corrélés au sein des habitats côtiers et entre ceux-ci. De plus, les valeurs de $\delta^{13}C$ des AA des otolithes fournissent un rapport entièrement alimentaire qui évite toute dilution par le carbone inorganique dissous. Les AA des otolithes servent de traceurs robustes des valeurs de $\delta^{13}C$ à la base du réseau alimentaire, ce qui fait de l'analyse SIA de composés spécifiques un outil puissant pour reconstituer les régimes alimentaires et pour suivre les déplacements des poissons le long d'isoscapes.

[Traduit par la Rédaction]

Introduction

The use of geochemical values in animal tissues as tags to track movement patterns of animals across isotope gradients in the environment (isoscapes) has become increasingly popular in terrestrial and aquatic systems (West et al. 2010). These studies have conducted bulk tissue stable isotope analyses (SIA) on a variety of tissues, including bird feathers, whale baleen, and fish scales (Hobson 1999; Rubenstein and Hobson 2004). Some of the most comprehensive examples of this approach have been conducted using fish otoliths to address questions of habitat residency, migration, and population connectivity (reviewed by Campana and Thorrold (2001) and Elsdon et al. (2008)). To date, studies have focused almost exclusively on the inorganic aragonite fraction of otoliths to provide information on the environment inhabited by individuals at different life history stages (Secor et al. 1995; Thorrold et al. 2001; Kennedy et al. 2002). Recent work has suggested that the bulk carbon isotope composition of otoliths may also record a significant dietary component

Received 15 July 2010. Accepted 26 March 2011. Published at www.nrcresearchpress.com/cjfas on 28 July 2011.
J21921

Paper handled by Associate Editor Bronwyn Gillanders.

K.W. McMahon. Massachusetts Institute of Technology and Woods Hole Oceanographic Institution, Joint Program in Oceanography and Ocean Engineering, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.
M.L. Fogel. Carnegie Institution of Washington, 5251 Broad Branch Road NW, Washington, DC 20015, USA.
B.J. Johnson. Department of Geology, Bates College, Lewiston, ME 04240, USA.
L.A. Houghton and S.R. Thorrold. Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.

Corresponding author: Kelton W. McMahon (e-mail: kmcmahon@whoi.edu).

Can. J. Fish. Aquat. Sci. **68**: 1330–1340 (2011) doi:10.1139/F2011-070 Published by NRC Research Press

(Elsdon et al. 2010). These results raise the intriguing possibility of using otolith geochemistry to retrospectively identify both lifetime movement patterns and diets of fishes.

Despite considerable promise, interpreting carbon isotope values in otoliths remains a difficult proposition. The carbon deposited in otoliths comes from both metabolic sources and dissolved inorganic carbon (DIC). These two potential sources have $\delta^{13}C$ values that may differ by as much as 20‰. Most studies have found that DIC contributes the majority of otolith carbon (Thorrold et al. 1997; Tohse and Mugiya 2004; Solomon et al. 2006), and therefore, dietary values in otoliths are inevitably diluted by DIC. More importantly, the relative contribution of these two end members is highly variable within and among species, making it difficult to mathematically correct for the DIC dilution effect. Indeed, variations in bulk otolith $\delta^{13}C$ appear to reflect a number of factors, including metabolism (Kalish 1991; Weidman and Millner 2000; Stephenson et al. 2001), diet $\delta^{13}C$ and trophic position (Gauldie 1996; Begg and Weidman 2001), DIC $\delta^{13}C$ (Schwarcz et al. 1998), and environmental conditions (Mulcahy et al. 1979; Kalish 1991). Therefore, it remains difficult to determine an unambiguous dietary $\delta^{13}C$ value from bulk analysis of otoliths.

One potential method for avoiding the confounding effect of DIC-derived carbon on otolith $\delta^{13}C$ values is to focus on otolith protein that may constitute up to 10% (by weight) of an otolith (Degens et al. 1969; Sasagawa and Mugiya 1996; Murayama et al. 2002). Analyzing otolith proteins may provide a purely dietary record that avoids the effect of both DIC dilution and variable metabolic carbon contribution. This protein value represents a mixture of amino acids (AAs) directly routed from dietary protein and AAs biosynthesized from a bulk carbon pool consisting of dietary proteins, lipids, and carbohydrates (Schwarcz 1991; Ambrose and Norr 1993; McMahon et al. 2010). Bulk protein SIA is not, however, without challenges. For instance, it can be difficult to distinguish between changes in $\delta^{13}C$ associated with diet or trophic shifts versus changes due to movement among habitats with different $\delta^{13}C$ values at the base of the food web ($\delta^{13}C_{Base}$ (Post 2002)). This is particularly true when tracking the ontogenetic shifts of highly migratory fishes, where juveniles and adults often occupy different habitats and different trophic levels (Cocheret de la Morinière et al. 2003; Graham et al. 2007).

Compound-specific SIA is a more powerful tool for examining diet and habitat use than conventional bulk SIA alone (Fantle et al. 1999; Popp et al. 2007; McMahon et al. 2010). Several studies have shown that bulk ^{13}C fractionation factors of 0‰ to 1‰ are underlain by little or no fractionation in essential AAs and fractionation factors of more than 7‰ in many non-essential AAs (Hare et al. 1991; Jim et al. 2006; McMahon et al. 2010). Although plants and bacteria can synthesize essential AAs de novo, most animals have lost the necessary enzymatic pathways to synthesize these AAs at a rate sufficient for normal growth (Borman et al. 1946; Reeds 2000). Therefore, essential AAs must be incorporated directly from the diet. As a result, $\delta^{13}C$ values of consumer essential AAs represent the isotopic signature of primary producers at the base of a food web ($\delta^{13}C_{Base}$) without the confounding influence of trophic fractionation. Conversely, non-essential AAs can either be synthesized de novo from a bulk carbon pool or directly incorporated from dietary protein into consumer tissue through isotopic routing (Schwarcz 1991). Non-essential AA $\delta^{13}C$ values may, therefore, provide valuable information about metabolic processing and have been shown to correlate with diet quality and composition (McMahon et al. 2010). Carbon isotope analysis of AAs has been applied to several other biominerals, including egg shells (Johnson et al. 1993, 1998), mollusk shells (Engel et al. 1994; Silfer et al. 1994; O'Donnell et al. 2007), bones (Hare et al. 1991; Howland et al. 2003), and teeth (Bada et al. 1990) to reconstruct past climates, examine diagenesis and mineral authenticity, and assess seasonal or ontogenetic shifts in consumer diet. Compound-specific SIA has also recently been applied to fish muscle to assess diet and habitat use (Popp et al. 2007; McMahon et al. 2010). However, researchers have yet to apply compound-specific SIA to accretionary tissues in fishes, including otoliths, that may allow for retrospective analyses of diet and movement.

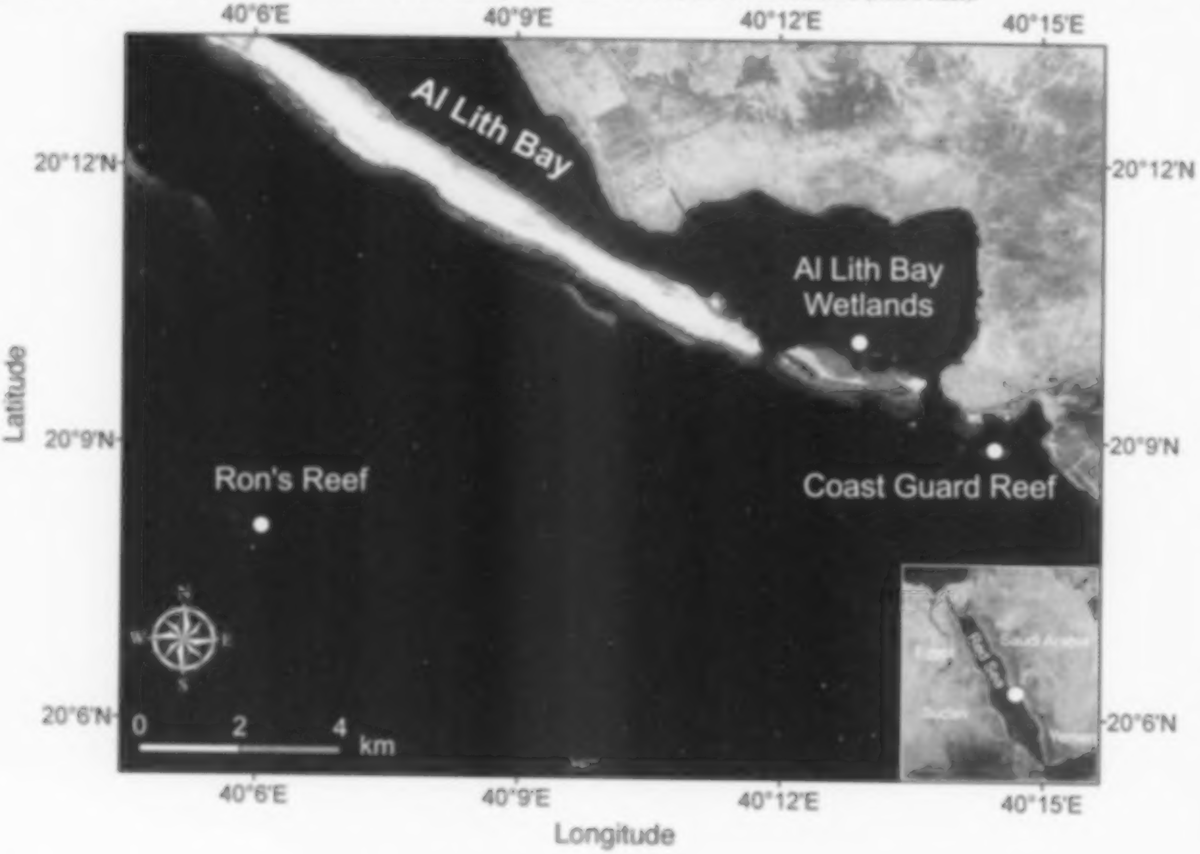
Here, we present a method for stable carbon isotope analysis of AAs in otoliths. To test the method, we compared $\delta^{13}C$ values of individual AAs in muscle tissue and otoliths from wild caught Ehrenberg's snapper, *Lutjanus ehrenbergii* (Peters, 1869), collected from three isotopically distinct habitats near Al Lith, Saudi Arabia, in the Red Sea. We hypothesized that the $\delta^{13}C$ values of otolith AAs would be strongly correlated with those of muscle AAs, providing access to dietary isotope values in otoliths that avoid the DIC dilution effect observed in bulk otolith SIA. We also hypothesized that otolith AA $\delta^{13}C$ values would provide a reliable tracer of residence in isotopically distinct habitats. Our study provides ecologists with a new tool for reconstructing dietary histories and establishing $\delta^{13}C_{Base}$ values to track fish movement through isoscapes.

Materials and methods

Field collections

Lutjanus ehrenbergii were collected at three locations along a cross-shelf transect from Al Lith, Saudi Arabia, in the Red Sea in March 2009 (Fig. 1). Associated with coral reefs as adults, juvenile *L. ehrenbergii* are abundant in coastal wetland habitats, making them a model species for examining residence along an isotopic gradient. Juvenile *L. ehrenbergii* (total length (TL) = 77 ± 6 mm) were collected from seagrass beds in Al Lith Bay (Al Lith Bay wetlands) using cast nets. Adult *L. ehrenbergii* were speared from a reef 2 km from the entrance of Al Lith Bay (Coast Guard Reef; TL = 209 ± 48 mm) and from a shelf reef approximately 14 km off the coast of Al Lith (Ron's Reef; TL = 232 ± 5 mm)). Sagittal otoliths and white muscle tissue were dissected from each fish in the field. Otoliths were cleaned of residual surface tissue with water and stored dry in 1.5 mL vials. White muscle samples from the dorsal surface of each fish were frozen on the boat prior to transport to an onshore laboratory. In the lab, white muscle samples were frozen at –20 °C and then lyophilized (freeze-dried) for 48 h. Paired otoliths and freeze-dried muscle samples were transferred back to Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA, for further preparation and analysis. Seventy-three fish, including 26 fish from Al Lith Bay wetlands, 23 fish from Coast Guard Reef, and 24

Fig. 1. Three locations for Ehrenberg's snapper, *Lutjanus ehrenbergii*, collected along a cross-shelf transect from Al Lith, Saudi Arabia, in the Red Sea in March 2009. Juvenile *L. ehrenbergii* were collected from seagrass beds in Al Lith Bay (Al Lith Bay wetlands), and adult *L. ehrenbergii* were collected from a reef adjacent to Al Lith Bay (Coast Guard Reef) and 14 km offshore (Ron's Reef).



fish from Ron's Reef, were analyzed for paired bulk otolith and muscle $\delta^{13}C$ values. Of those fish, five were randomly selected per location for paired compound-specific SIA of otoliths and muscle. Percentage protein for *L. ehrenbergii* muscle and otoliths was determined at the New Jersey Feed Laboratory, Trenton, New Jersey, USA (AOAC Method 994.12; AOAC International 2005).

Sample preparation

A single sagittal otolith was randomly selected from each fish. All otolith samples were scrubbed and rinsed in ultrapure water, cleaned ultrasonically for 5 min in ultrapure water, and then air-dried under a class-100 positive-flow fume hood for 24 h. Whole otoliths from juvenile *L. ehrenbergii* were used for SIA. For adult *L. ehrenbergii*, we extracted otolith powder after the last annulus of the whole otolith, corresponding to the most recent several months of growth, using a Merchantek MicroMill (Electro Scientific Industries, Portland, Oregon, USA) to provide the closest temporal match possible between muscle and otolith material. Otolith powder was milled onto weigh paper and weighed to the nearest microgram.

Otolith material was homogenized with a mortar and pestle and then subdivided into two portions for bulk inorganic and compound-specific SIA. Approximately 50 μg of otolith material was transferred to a glass reaction vessel and analyzed for bulk $\delta^{13}C$ on a Thermo Finnigan Mat 253 isotope ratio monitoring mass spectrometer (irm-MS) with a Kiell III carbonate device at Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA, following the methods of Ostermann and Curry (2000). External precision of the mass spectrometer for $\delta^{13}C$ measurements in carbonate standards was ±0.03‰ (Ostermann and Curry 2000). Approximately 10 mg of otolith material from each fish was processed to isolate individual AAs. Samples of homogenized otolith powder were acid-hydrolyzed in 4 mL Teflon-lined screw cap vials along with 0.1 mL of 6 $mol \cdot L^{-1}$ HCl per milligram of otolith under a N_2 atmosphere at 110 °C for 20 h. Samples were neutralized with ultrapure water and evaporated to dryness under a gentle stream of N_2 to remove HCl. Samples were stored frozen until they were derivatized just prior to compound-specific SIA.

Freeze-dried, homogenized white muscle samples from each fish were also subdivided into two portions. Approximately 1 mg of muscle was weighed into a tin cup and analyzed for bulk $\delta^{13}C$ values using a Europa Hydra 20/20 irm-

MS at the UC Davis Stable Isotope Facility, Davis, California, USA. A second portion of each sample (~500 μg) was acid-hydrolyzed with 1 mL of 6 mol·L⁻¹ HCl per milligram of freeze-dried muscle tissue as described previously for the otolith samples. Dried, neutralized samples were also stored frozen until derivatization.

Compound-specific stable isotope analysis

Amino acids contain highly polar functional groups (e.g., carboxylic acid groups), making them difficult to analyze by gas chromatography – combustion – isotope ratio monitoring mass spectrometry (GC-C-irm-MS) (reviewed by Klee 1985). As a result, amino acids must first be derivatized through the addition of less polar functional groups. Acid-hydrolyzed otolith and muscle samples were therefore derivatized prior to analysis using an approach modified from Silfer et al. (1991) and Johnson et al. (1998). All reactions were performed with analytical grade reagents in muffled glassware to prevent contamination. First, each sample underwent an acid-catalyzed esterification. Each sample vial received 0.8 mL of a 2-propanol and acetyl chloride solution (4:1 by volume). Vials were put under an atmosphere of N_2 and placed on a heating block at 110 °C for 1 h. The reactions were then quenched in an ice bath, and the otolith samples were quantitatively transferred to new 4 mL vials using dichloromethane (DCM), leaving behind salts associated with the acid hydrolysis of carbonate. All samples were dried under a gentle stream of N_2. To remove any remaining acidified isopropanol, samples were brought up in 0.5 mL of DCM and dried under N_2 three times. Samples were then acylated with 0.5 mL of trifluoroacetic anhydride (TFAA) and 0.5 mL of DCM under an atmosphere of N_2 at 110 °C for 10 min. Again, reactions were quenched in an ice bath, and excess TFAA was removed as described above using three rinses of DCM.

The derivatization process alters the $\delta^{13}C$ values of sample AAs through addition of exogenous carbon associated with the added functional groups and kinetic fractionation associated with the derivatization reactions (Rieley 1994; Docherty et al. 2001). An AA standard, consisting of all AAs of interest, was created from individual AAs (Sigma-Aldrich Co., St. Louis, Missouri, USA). The $\delta^{13}C$ value of each AA was determined via elemental analyzer irm-MS at Woods Hole Oceanographic Institution prior to creating the composite AA standard. The AA standard was concurrently derivatized with each batch of samples. Derivatization correction factors were determined for each AA of interest based on the known $\delta^{13}C$ value of the AAs in the standard prior to derivatization and the postderivatization $\delta^{13}C$ values determined with each sample batch. The correction factors were applied to each sample to adjust for the introduction of exogenous carbon and kinetic fractionation during derivatization.

Derivatized samples were brought up in DCM and injected on column in splitless mode at 260 °C and separated on an HP Ultra-1 column (50 m length, 0.32 mm inner diameter, and 0.5 μm film thickness; Hewlett Packard, Wilmington, Delaware, USA) in an Agilent 6890N Gas Chromatograph (GC) at Woods Hole Oceanographic Institution. Sample concentrations were adjusted to achieve a minimum 2 V output for all AAs. Gas chromatography conditions were set to optimize peak separation and shape as follows: initial temperature 75 °C held for 2 min; ramped to 90 °C at 4 °C·min⁻¹, held for 4 min; ramped to 185 °C at 4 °C·min⁻¹, held for 5 min; ramped to 250 °C at 10 °C·min⁻¹, held 2 min; ramped to 300 °C at 20 °C·min⁻¹, held for 8 min. The separated AA peaks were combusted online in a Finnigan GC-C continuous flow interface at 930 °C and then measured as CO_2 on a Thermo Finnigan Mat 253 irm-MS. Standardization of runs was achieved using intermittent pulses of a CO_2 reference gas of known isotopic composition. All compound-specific SIA samples were analyzed in duplicate along with AA standards of known isotopic composition. The glutamic acid and aspartic acid peaks contained unknown contributions from glutamine and asparagine, respectively, due to conversion to their dicarboxylic acids during acid hydrolysis. The relative abundance (%) of individual AAs in otoliths and muscle were calculated from mass 44 peak area based on standards of known concentration.

Data analysis

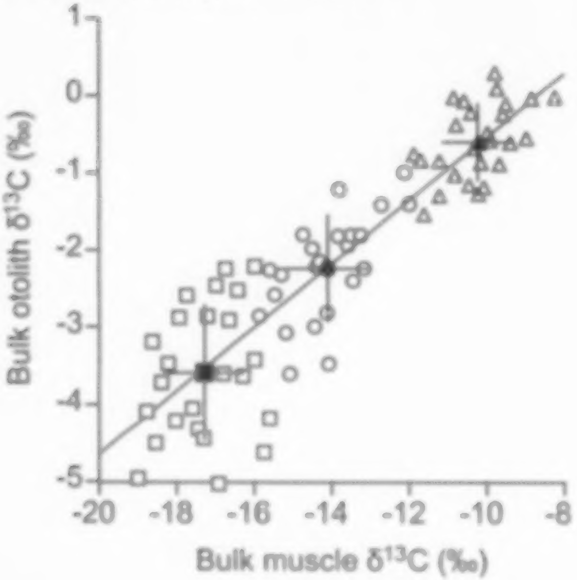
Stable isotope ratios were expressed in standard delta (δ) notation:

$$\delta^{13}C_{\text{sample}} = \left(\frac{{}^{13}C/{}^{12}C_{\text{sample}}}{{}^{13}C/{}^{12}C_{\text{std}}} \right) \times 1000$$

where the standard for carbon was Vienna PeeDee Belemnite (VPDB). We determined the relationship between paired bulk muscle and otolith $\delta^{13}C$ values from 73 fish collected at the three sites using linear regression. We tested for differences in $\delta^{13}C$ of bulk muscle and otoliths among sites using separate one-way analyses of variance (ANOVAs) and Tukey's honestly significant difference (HSD) post-hoc tests (α = 0.05). Bulk muscle and otolith data met the assumptions of normality and equality of variances. The relationships between $\delta^{13}C$ values of individual AAs from paired otolith and muscle samples were determined by linear regression analyses for each AA (n = 15 fish per AA). Differences in $\delta^{13}C$ values of non-essential and essential AAs in *L. ehrenbergii* otoliths among sites were determined using separate multivariate ANOVAs (MANOVA; α = 0.05). We assigned AAs as non-essential or essential according to Karasov and Martínez del Rio (2007).

Minimum sample sizes necessary for compound-specific SIA of otolith and muscle were determined by extrapolation of sample sizes used in this study down to the GC-C-irm-MS lower limit of detection for the least abundant AAs. Three aliquots of the same AA standard were derivatized at the same time under the same reaction conditions (for within-batch variability) on three separate days (for among-batch variability). To examine the variability in AA $\delta^{13}C$ values within and among derivatization batches, the mean relative standard deviation (RSD) within batch and among batch was calculated across all 11 AAs. The desktop stability of derivatives was assessed by analyzing three aliquots of the same AA standard a total of 20 times each over the course of nine days. Overall external precision of the $\delta^{13}C$ measurements after correcting for the fractionation associated with derivatization was 0.80‰ ± 0.96‰ (standard deviation (SD)), averaged across all AAs.

Fig. 2. Linear relationship between bulk otolith and bulk muscle $\delta^{13}C$ values from *Lutjanus ehrenbergii* collected from three isotopically distinct habitats near Al Lith, Saudi Arabia, in the Red Sea ($n = 73$ fish; open symbols), including mean (± standard deviation (SD); solid symbols) muscle and otolith $\delta^{13}C$ values for Al Lith Bay wetlands (triangles; $n = 26$ fish), Coast Guard Reef (circles, $n = 23$ fish), and Ron's Reef (squares; $n = 24$ fish).



Results

We found a significant linear relationship between paired bulk muscle and otolith $\delta^{13}C$ values of *L. ehrenbergii* (linear regression, $y = 0.38x + 3.31$, $R^2 = 0.83$; Fig. 2). *Lutjanus ehrenbergii* collected from three locations near Al Lith, Saudi Arabia, had distinct $\delta^{13}C$ values for bulk otoliths (one-way ANOVA, df = 2, 14, $F = 13.3$, $p < 0.05$) and bulk muscle (one-way ANOVA, df = 2, 14, $F = 58.9$, $p < 0.05$). However, *L. ehrenbergii* from Coast Guard Reef and Ron's Reef had statistically similar otolith $\delta^{13}C$ values, with $p < 0.05$ for all other pairwise comparisons. The overall range in $\delta^{13}C$ values among locations was much larger for bulk muscle (7.2‰) than it was for bulk otolith (2.6‰). Individuals from seagrass habitats in the Al Lith Bay wetlands had the most positive $\delta^{13}C$ values for muscle (mean ± SD = −10.3‰ ± 1.0‰) and otoliths (−0.7‰ ± 0.6‰), whereas fish from the offshore reef had the most negative $\delta^{13}C$ values for muscle (−17.5‰ ± 0.8‰) and otoliths (−3.3‰ ± 1.1‰). Fish from the reef adjacent to Al Lith Bay had intermediate $\delta^{13}C$ values for muscle (−14.6‰ ± 1.2‰) and otoliths (−2.3‰ ± 0.6‰).

Protein comprised 0.6% ± 0.1% of otoliths and 92.3% ± 1.3% of muscle for *L. ehrenbergii* ($n = 3$). Eleven individual AAs from muscle and otolith protein with sufficient peak size and GC baseline resolution were identified and analyzed for $\delta^{13}C$ via GC-C-irm-MS (Fig. 3). Glutamic acid and aspartic acid were the most abundant AAs in *L. ehrenbergii* muscle and otolith, whereas leucine and threonine were the most abundant essential AAs in muscle and otolith (Table 1). Based on the least abundant AA in our analyses, isoleucine for otoliths and proline for muscle, and the lower limit of detection for the MAT 253 irm-MS, the minimum sample size needed to conduct compound-specific SIA on *L. ehrenbergii* was 500–1000 µg for otoliths and 10–15 µg for muscle. The derivatization process made the $\delta^{13}C$ values of the AAs in the standard more negative, although the shifts were not uniform among AAs. Variability in the $\delta^{13}C$ values of derivatized AA standards was much smaller within derivatization batches (mean RSD = 0.8% ± 0.2% SD) than among batches (2.2% ± 1.6% SD). Repeated injections of the same derivatized standard were very consistent, showing low variability in $\delta^{13}C$ values (mean SD for all AAs = 0.35‰ ± 0.14‰ SD) for the first 160 h after derivatization (Fig. 4). After approximately 160 h, the $\delta^{13}C$ values of AAs in the standard became significantly more variable (1.25‰ ± 0.57‰) and tended to become more positive with time. The shift was not consistent across all AAs, as serine and threonine typically became unstable first. Similar patterns were also observed in the fish muscle and otolith samples.

We found a strong linear relationship between individual otolith and muscle AA $\delta^{13}C$ values (linear regression, $y = (0.84 \pm 0.25)x - (1.72 \pm 3.66)$, $R^2 = 0.70 \pm 0.22$) of *L. ehrenbergii* (Fig. 5). Otolith $\delta^{13}C$ values in AAs generally tracked the patterns observed in the bulk muscle and otoliths, although the otolith AA $\delta^{13}C$ range was closer to the bulk muscle range, particularly for several of the essential AAs (Fig. 6). Individual AAs from otoliths of fish collected in the Al Lith Bay wetlands typically had the most positive $\delta^{13}C$ values, and those from the offshore reef often had the most negative $\delta^{13}C$ values, with otolith AAs of fish collected in Coast Guard Reef intermediate (Fig. 6). We found significant differences in otolith $\delta^{13}C$ values for non-essential AAs (MANOVA, Pillai's trace = 0.97, df = 6, 8, $F = 48.04$, $p < 0.05$) and essential AAs (MANOVA, pillai trace = 0.92, df = 5, 9, $F = 21.37$, $p < 0.05$).

Discussion

Stable isotope analysis (SIA) of AAs in otolith protein provides a new way to retrospectively address questions of diet, habitat use, and migration in fishes. The method avoids many of the complications associated with conventional bulk SIA of fish otoliths, including DIC dilution of dietary signatures and variable metabolic carbon contribution to otolith $\delta^{13}C$ values. We tested this new approach by sampling muscle and otoliths from Ehrenberg's snapper, *L. ehrenbergii*, collected along a carbon isotope gradient from coastal seagrass habitats to offshore coral reefs in the Red Sea. Fish from Al Lith Bay wetlands had the most positive muscle $\delta^{13}C$ values (−10.4‰), which likely reflected the carbon contribution of seagrasses within the bay. Seagrasses are C4 primary producers at the base of the food web, with $\delta^{13}C$ values between −8‰ and −12‰ (Hemminga and Mateo 1996). In contrast, *L. ehrenbergii* muscle tissue from the reef 14 km offshore had the most negative $\delta^{13}C$ values (−17.5‰), reflecting a marine phytoplankton $\delta^{13}C_{Base}$ signature that typically ranges from −17‰ to −21‰ (Descolas-Gros and Fontungne 1990). Fish from the reef adjacent to Al Lith Bay had intermediate $\delta^{13}C$ values for muscle (−14.6‰) that presumably indicated carbon inputs from both seagrasses and phytoplankton sources. The observed $\delta^{13}C$ isoscape proved an ideal system to test

Fig. 3. A representative gas chromatogram of derivatized individual amino acids from an otolith of *Lutjanus ehrenbergii*. CO_2 ref, intermittent pulses of a CO_2 gas reference of known isotopic composition. Amino acids: Gly, glycine; Ser, serine; Asp, aspartic acid; Glu, glutamic acid; Pro, proline; Ala, alanine; Thr, threonine; Ile, isoleucine; Val, valine; Phe, phenylalanine; Leu, leucine.

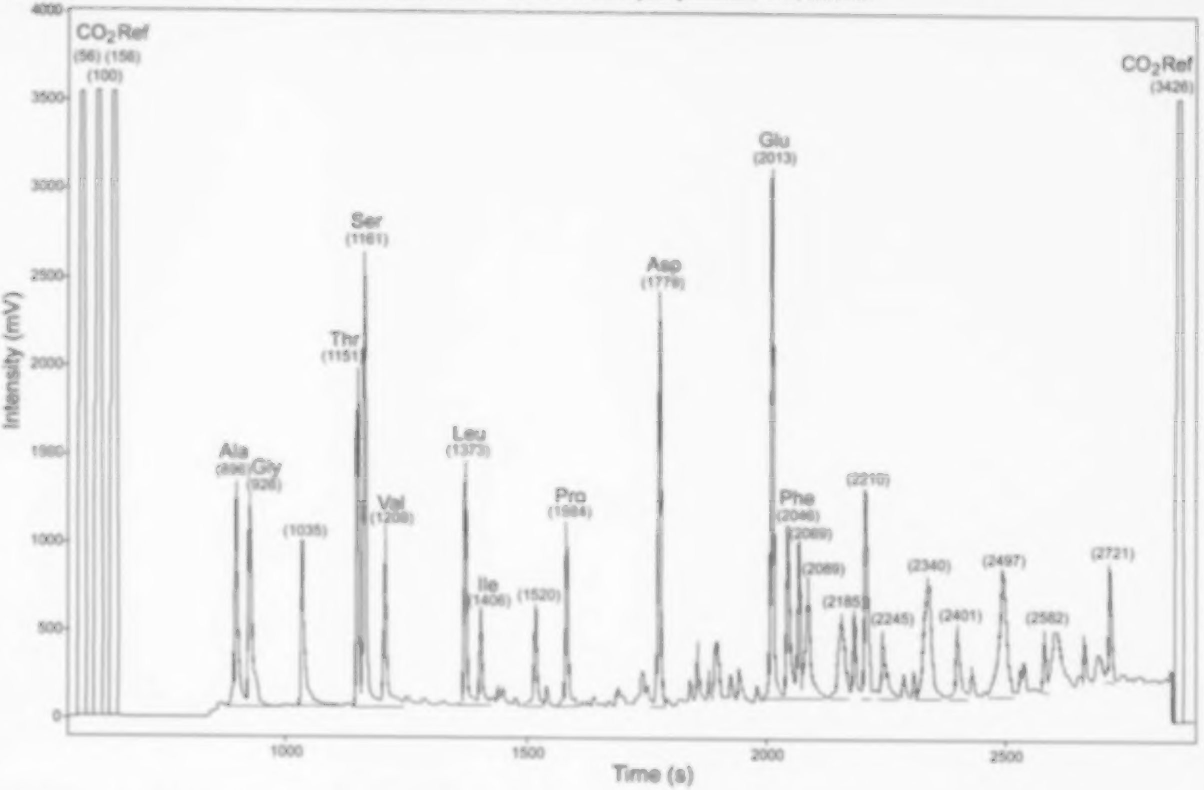


Table 1. The relative abundance (mean percentage ± standard deviation (SD)) of 11 individual amino acids in otolith and muscle of *Lutjanus ehrenbergii* calculated from mass 44 peak area and standards of known concentration via gas chromatography.

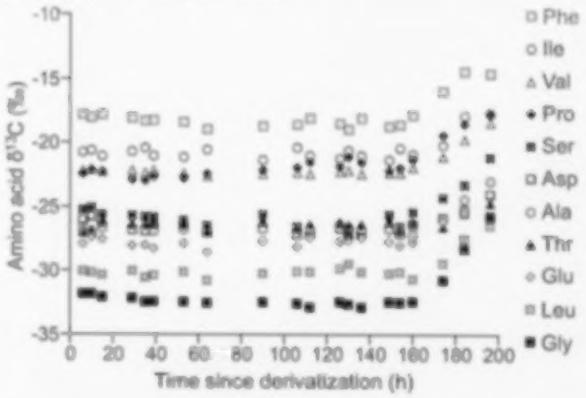
Amino acids	Otolith	Muscle
Non-essential		
Gylcine	7.2±0.6	5.8±0.2
Serine	9.2±0.5	5.1±0.6
Aspartic acid	16.5±1.0	16.6±0.3
Glutamic acid	27.0±1.2	26.1±1.2
Proline	9.8±1.5	4.6±2.5
Alanine	6.6±0.8	7.5±0.3
Essential		
Threonine	6.6±0.8	5.4±0.1
Isoleucine	3.2±0.9	5.1±0.1
Valine	4.0±0.3	5.6±0.2
Phenylalanine	4.0±0.3	5.3±0.1
Leucine	5.9±0.5	13.0±0.4
Percentage protein	0.6±0.1	92.2±1.3

Note: For relative abundance, *n* = three sites with five fish per site; for percentage protein of otolith and muscle, *n* = three fish.

the ability of AAs in otoliths to accurately record $\delta^{13}C_{\mathrm{Base}}$ signatures compared with conventional bulk otolith SIA.

Bulk otolith $\delta^{13}C$ values were significantly different among the three habitats and significantly correlated with bulk muscle $\delta^{13}C$ values. Several recent studies have suggested that bulk otolith records some dietary information even though most of the carbon comes from DIC in the water column (Dufour et al. 2007; Mateo 2009; Elsdon et al. 2010).

Fig. 4. $\delta^{13}C$ values of derivatized amino acid standards analyzed 20 times over the course of nine days via gas chromatography – combustion – isotope ratio monitoring – mass spectrometry. Amino acids: Gly, glycine; Ser, serine; Asp, aspartic acid; Glu, glutamic acid; Pro, proline; Ala, alanine; Thr, threonine; Ile, isoleucine; Val, valine; Phe, phenylalanine; Leu, leucine.



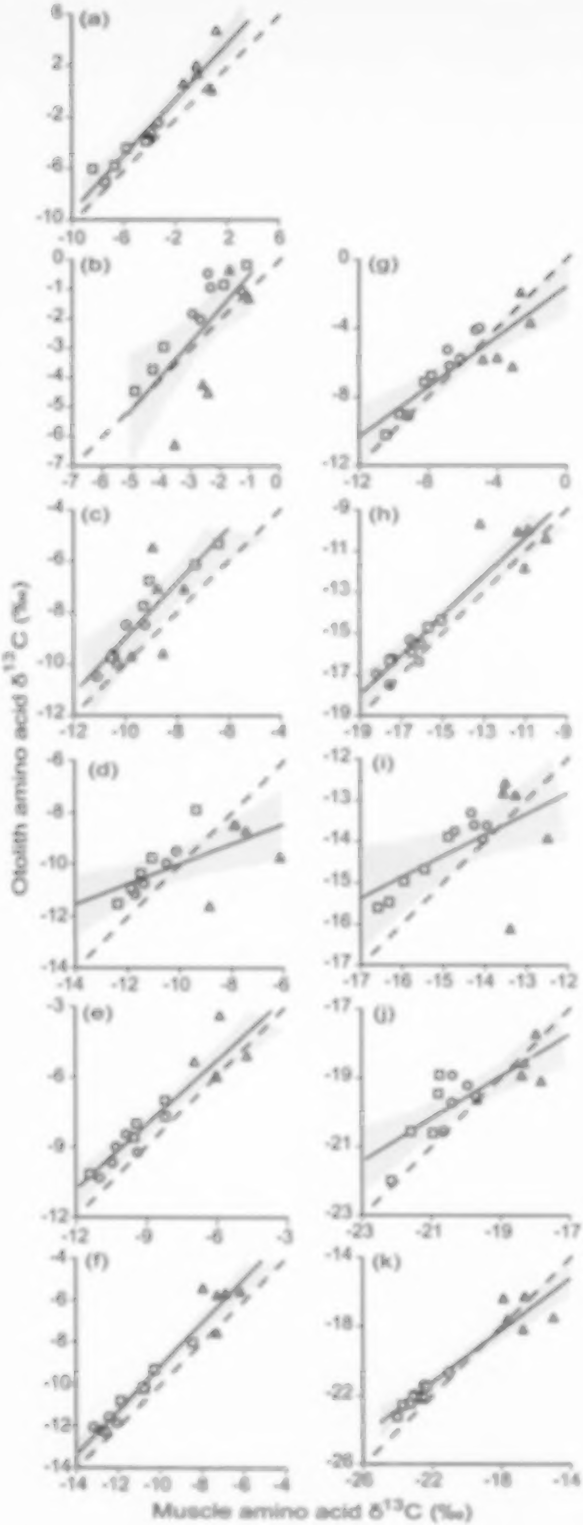
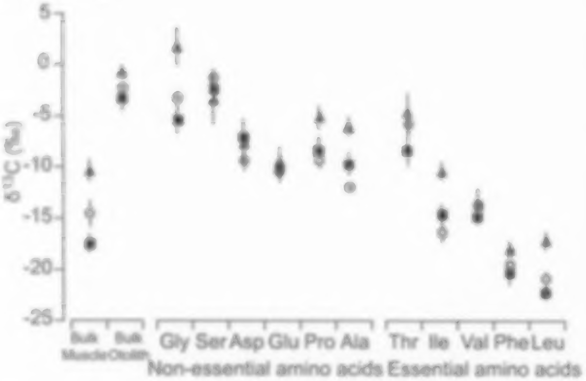


Fig. 5. Linear relationships (solid line with shaded 95% confidence intervals) of paired otolith and muscle amino acid $\delta^{13}C$ values for 11 individual amino acids from *Lutjanus ehrenbergii* collected from Al Lith Bay wetlands (triangles), Coast Guard Reef (circles), and Ron's Reef (squares) near Al Lith, Saudi Arabia, in the Red Sea (n = 15 fish). Panels *a* to *f* represent non-essential amino acids: (*a*) glycine, (*b*) serine, (*c*) aspartic acid, (*d*) glutamic acid, (*e*) proline, and (*f*) alanine. Panels *g* to *k* represent essential amino acids: (*g*) threonine, (*h*) isoleucine, (*i*) valine, (*j*) phenylalanine, (*k*) leucine. Broken line represents the 1:1 line. Scales are not the same among panels.

Fig. 6. Bulk muscle and otolith $\delta^{13}C$ values (mean ± SD) and otolith non-essential and essential amino acid $\delta^{13}C$ values (mean ± SD) from *Lutjanus ehrenbergii* collected from three isotopically distinct habitats: Al Lith Bay wetlands (triangles), Coast Guard Reef (circles), and Ron's Reef (squares) near Al Lith, Saudi Arabia, in the Red Sea (n = five fish per site). Amino acids: Gly, glycine; Ser, serine; Asp, aspartic acid; Glu, glutamic acid; Pro, proline; Ala, alanine; Thr, threonine; Ile, isoleucine; Val, valine; Phe, phenylalanine; Leu, leucine.



However, this variability in bulk otolith $\delta^{13}C$ among sites may have been due to a number of factors, including changes in metabolic rate with ontogeny (Kalish 1991; Weidman and Millner 2000; Stephenson et al. 2001), changes in diet $\delta^{13}C$ and trophic position shifts between juvenile and adult fish (Gauldie 1996; Begg and Weidman 2001), changes in seawater DIC $\delta^{13}C$ with distance offshore (Schwarcz et al. 1998), or other environmental conditions (Mulcahy et al. 1979; Kalish 1991). Although there were significant differences in bulk otolith $\delta^{13}C$ values across habitats, it remains difficult to determine an unambiguous dietary $\delta^{13}C$ value from bulk $\delta^{13}C$ analysis of otoliths. Furthermore, otolith values recorded less than half of the $\delta^{13}C$ range seen in muscle tissue. As a result, the ability to discriminate among habitats using the bulk otolith data was reduced compared with muscle $\delta^{13}C$ values. This was particularly true when comparing fish from Coast Guard Reef and Ron's Reef, where muscle $\delta^{13}C$ values showed significantly larger differences between habitats (mean difference = 3‰) than otolith $\delta^{13}C$ values (mean difference = 1‰).

Muscle AA $\delta^{13}C$ values accounted for most of the observed variation in otolith AA $\delta^{13}C$ values with a slope of the relationship near to 1. Otolith AAs, therefore, recorded nearly identical dietary information to that of muscle AAs.

This is perhaps not surprising considering both muscle and otoliths likely receive AAs for protein synthesis from a common AA pool in the blood. Our results also suggest that any fractionation during transport of AAs from blood to the site of protein synthesis and subsequent release from the macula (Murayama 2000; Murayama et al. 2004) was minor. As such, δ^{13}C analysis of otolith AAs should provide an archival record of fish diet that has previously been inaccessible with conventional inorganic otolith analysis.

Stable isotope analysis of individual AAs has greatly improved the study of diet (Fantle et al. 1999; Fogel and Tuross 2003), habitat use (Popp et al. 2007), and the sources of complex mixtures of organic matter (Uhle et al. 1997; McCarthy et al. 2004) for a number of terrestrial and aquatic taxa. It can be challenging with bulk SIA to distinguish differences in consumer δ^{13}C due to changes in trophic level versus changes in $\delta^{13}C_{Base}$. Most recently, in a controlled feeding experiment, McMahon et al. (2010) showed that essential AAs in fish muscle recorded the δ^{13}C values of diet with little or no trophic fractionation, thereby providing an accurate record of $\delta^{13}C_{Base}$ signature. We found that juvenile *L. ehrenbergii* in Al Lith Bay wetlands had essential AA δ^{13}C signatures that were very different from adult *L. ehrenbergii* on coastal and shelf reefs. The distinction was clear even when comparing *L. ehrenbergii* from Al Lith Bay wetlands and Coast Guard Reef, which were only 2 km apart. The unique habitat signatures in coastal wetlands and coral reefs can be traced to the local food web isotope values in those habitats (McMahon 2011). Our study suggests that otolith essential AA SIA should be particularly valuable for retrospectively assessing residence in and movement among habitats with distinct food web $\delta^{13}C_{Base}$ values.

O'Donnell et al. (2007) studied δ^{13}C values of AAs from modern and fossil *Mercenaria* shells to examine preservation of AAs in biominerals and regional and ontogenetic variability in δ^{13}C values. The authors concluded that the range of δ^{13}C values in AAs from modern *Mercenaria* collected from coastal Virginia and coastal Florida suggested the preservation of a dietary signal in the bivalve shells. In addition, the authors showed significant variation in *Mercenaria* shell AA δ^{13}C values within and among years, likely reflecting a shift in the relative contribution of primary producers at the base of the food web. Similar work by Johnson et al. (1998) provides another example of using AA SIA of biominerals to examine diet and local habitat use. These authors showed that AA δ^{13}C values from ostrich egg shells reflected the diet of ostrich at the time of egg formation and could identify local climate and vegetation conditions. Our data, in concert with these studies, suggest that compound-specific SIA of otoliths is a valuable approach to determining residence in habitats with distinct $\delta^{13}C_{Base}$ signatures.

In addition to determining $\delta^{13}C_{Base}$ signatures, AA δ^{13}C values in otoliths may record other valuable information about diet that was previously difficult to assess with conventional bulk otolith SIA. McMahon et al. (2010) found that δ^{13}C values of non-essential AAs in fish muscle showed diet-specific patterns of de novo biosynthesis and direct isotopic routing from dietary protein. The AA composition and lipid to carbohydrate ratio of the diet both appeared to play a role in determining the δ^{13}C value of muscle non-essential AAs. In our study, otolith non-essential AAs showed similar patterns to essential AAs, suggesting that they recorded similar shifts in $\delta^{13}C_{Base}$ among habitats. This observation suggests a high degree of direct isotopic routing of non-essential AAs from dietary protein into fish biomass. Previous research has shown that consumers feeding on high protein diets often route most AAs, including non-essentials, directly from diet as a means of energy conservation, because dietary routing is typically more efficient than de novo biosynthesis (Ambrose and Norr 1993; Tieszen and Fagre 1993; Jim et al. 2006). Although the mechanisms driving non-essential AA δ^{13}C values remain unknown, the findings of McMahon et al. (2010) suggest that valuable dietary information is stored in δ^{13}C values of non-essential AAs. Given the high correlation between muscle and otolith AA δ^{13}C values, it is likely that the otolith non-essential AAs will also provide valuable information about diet and food quality.

We calculated a minimum sample size of 500–1000 μg for AA δ^{13}C analysis of *L. ehrenbergii* otoliths. Although *L. ehrenbergii* have relatively large otoliths, the percentage organic matter in these otoliths is quite small (~0.6%). There is significant variability in the percentage organic matter in otoliths that may vary by as much as 10% depending on species and life history stage (Degens et al. 1969; Morales-Nin 1986; Jolivet et al. 2008). As a result, the minimum sample size necessary for compound-specific SIA of *L. ehrenbergii* otoliths is likely significantly larger than for many other species. The temporal resolution of our approach will depend largely on the size and organic content in otoliths of the study species. As instrument sensitivity improves and sample size requirements decrease, we remain confident that we will be able to address questions of diet and movement at higher temporal resolution and on smaller otoliths. The sample size necessary for compound-specific analyses using GC-C-irm-MS may never be as small as that necessary for bulk otolith δ^{13}C measurements; however, δ^{13}C values of otolith AAs provide a wealth of information not available from conventional bulk analyses.

In conclusion, δ^{13}C analysis of AAs in otoliths is a valuable new tool for retrospective analysis of diet and movement patterns of fishes. Otolith AA δ^{13}C signatures were highly correlated with muscle values and provided a purely dietary value that avoided the confounding factors of DIC dilution and variable metabolic carbon contribution found in bulk otolith δ^{13}C analysis. The otolith AA approach provides access to dietary information in otoliths that should compliment data from conventional bulk otolith SIA. As with any stable isotope study of diet and movement, if fish reside in isotopically indistinguishable habitats or move through habitats faster than the habitat signature is recorded, then the method that we describe here may not identify residence in all intermediate habitats. However, otolith essential AAs provide a valuable tracer of residence in isotopically distinct habitats that increases our ability to track the movement of migratory fish or determine the $\delta^{13}C_{Base}$ values for resident individuals. Conversely, non-essential AAs may provide important archived information about diet composition and metabolic processing. Further research will, however, be needed before we can confidently interpret the biological significance of δ^{13}C variations in non-essential AAs in otoliths or other tissues.

Acknowledgements

The authors thank M. Berumen for field assistance and the crew of Dream Divers, Jeddah, Saudi Arabia, for boat and dive operation assistance. The site map was created by C. Braun. This research was based on work supported by awards USA 00002 and KSA 00011 made by King Abdullah University of Science and Technology (KAUST). Additional funding was provided by Woods Hole Oceanographic Institution and an International Society for Reef Studies – Ocean Conservancy Coral Reef Fellowship. K. McMahon received support from the National Science Foundation Graduate Research Fellowship Program.

References

Ambrose, S.H., and Norr, L. 1993. Carbon isotopic evidence for routing of dietary protein to bone collagen, and whole diet to bone apatite carbonate: purified diet growth experiments. *In* Molecular archaeology of prehistoric human bone. *Edited by* J. Lambert and G. Grupe. Springer-Verlag, Berlin, Germany. pp. 1–37.

AOAC International. 2005. Official methods of analysis. 17th ed. AOAC International, Arlington, Virginia.

Bada, J.L., Peterson, R.O., Schimmelmann, A., and Hedges, R.E.M. 1990. Moose teeth as monitors of environmental isotopic parameters. Oecologia (Berl.), **82**(1): 102–106. doi:10.1007/BF00318540.

Begg, G.A., and Weidman, C.R. 2001. Stable $\delta^{13}C$ and $\delta^{18}O$ isotopes in otoliths of haddock *Melanogrammus aeglefinus* from the northwest Atlantic Ocean. Mar. Ecol. Prog. Ser. **216**: 223–233. doi:10.3354/meps216223.

Borman, A., Wood, T.R., Black, H.C., Anderson, E.G., Oesterling, M.J., Womack, M., and Rose, W.C. 1946. The role of arginine in growth with some observations on the effects of argininic acid. J. Biol. Chem. **166**(2): 585–594. PMID:20276173.

Campana, S.E., and Thorrold, S.R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. **58**(1): 30–38. doi:10.1139/f00-177.

Cocheret de la Morinière, E.C., Pollux, B.J.A., Nagelkerken, I., and van der Velde, G. 2003. Diet shifts of Caribbean grunts (Haemulidae) and snappers (Lutjanidae) and the relation with nursery-to-coral reef migration. Estuar. Coast. Shelf Sci. **57**(5–6): 1079–1089. doi:10.1016/S0272-7714(03)00011-8.

Degens, E.T., Deuser, W.G., and Haedrich, R.L. 1969. Molecular structure and composition of fish otoliths. Mar. Biol. (Berl.), **2**(2): 105–113. doi:10.1007/BF00347005.

Descolas-Gros, C., and Fontungne, M. 1990. Stable carbon isotope fractionation by marine phytoplankton during photosynthesis. Plant Cell Environ. **13**(3): 207–218. doi:10.1111/j.1365-3040.1990.tb01305.x.

Docherty, G., Jones, V., and Evershed, R.P. 2001. Practical and theoretical considerations in the gas chromatography/combustion/isotope ratio mass spectrometry $\delta^{13}C$ analysis of small polyfunctional compounds. Rapid Commun. Mass Spectrom. **15**(9): 730–738. doi:10.1002/rcm.270. PMID:11319796.

Dufour, E., Gerdeaux, D., and Wurster, C.M. 2007. Whitefish (*Coregonus lavaretus*) respiration rate governs intra-otolith variation of $\delta^{13}C$ values in Lake Annecy. Can. J. Fish. Aquat. Sci. **64**(12): 1736–1746. doi:10.1139/f07-132.

Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Secor, D.H., Thorrold, S.R., and Walther, B.D. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. Annu. Rev. **46**: 297–330.

Elsdon, T.S., Ayvazian, S., McMahon, K.W., and Thorrold, S.R. 2010. Experimental evaluation of stable isotope fractionation in fish muscle and otoliths. Mar. Ecol. Prog. Ser. **408**: 195–205. doi:10.3354/meps08518.

Engel, M.H., Goodfriend, G., Qian, Y., and Macko, S. 1994. Indigeneity of organic matter in fossils: a test using stable isotope analysis of amino acid enantiomers in Quaternary mollusk shells. Proc. Natl. Acad. Sci. U.S.A. **91**(22): 10475–10478. doi:10.1073/pnas.91.22.10475. PMID:7937978.

Fantle, M.S., Dittel, A.I., Schwalm, S.M., Epifanio, C.E., and Fogel, M.L. 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia (Berl.), **120**(3): 416–426. doi:10.1007/s004420050874.

Fogel, M.L., and Tuross, N. 2003. Extending the limits of paleodietary studies of humans with compound specific carbon isotope analysis of amino acids. J. Archaeol. Sci. **30**(5): 535–545. doi:10.1016/S0305-4403(02)00199-1.

Gauldie, R.W. 1996. Biological factors controlling the carbon isotope record in fish otoliths: principles and evidence. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **115**(2): 201–208. doi:10.1016/0305-0491(96)00077-6.

Graham, B.S., Grubbs, D., Holland, K., and Popp, B.N. 2007. A rapid ontogenetic shift in the diet of juvenile yellowfin tuna from Hawaii. Mar. Biol. (Berl.), **150**(4): 647–658. doi:10.1007/s00227-006-0360-y.

Hare, P.E., Fogel, M.L., Stafford, T.W., Mitchell, A.D., and Hoering, T.C. 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J. Archaeol. Sci. **18**(3): 277–292. doi:10.1016/0305-4403(91)90066-X.

Hemminga, M.A., and Mateo, M.A. 1996. Stable carbon isotopes in seagrass: variability in ratios and use in ecological studies. Mar. Ecol. Prog. Ser. **140**: 285–298. doi:10.3354/meps140285.

Hobson, K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia (Berl.), **120**(3): 314–326. doi:10.1007/s004420050865.

Howland, M.R., Corr, L.T., Young, S.M., Jones, V., Jim, S., Van der Merwe, N.J., Mitchell, A.D., and Evershed, R.P. 2003. Expression of the dietary isotope signal in the compound-specific $\delta^{13}C$ values of pig bone lipids and amino acids. Int. J. Osteoarchaeol. **13**(1–2): 54–65. doi:10.1002/oa.658.

Jim, S., Jones, V., Ambrose, S.H., and Evershed, R.P. 2006. Quantifying dietary macronutrient sources of carbon for bone collagen biosynthesis using natural abundance stable carbon isotope analysis. Br. J. Nutr. **95**(6): 1055–1062. doi:10.1079/BJN20051685. PMID:16768826.

Johnson, B.J., Fogel, M.L., and Miller, G.H. 1993. Paleoecological reconstructions in southern Egypt based of the stable carbon and nitrogen isotopes in the organic fraction and stable carbon isotopes in individual amino acids of fossil ostrich eggshell. Chem. Geol. **107**(3–4): 493–497. doi:10.1016/0009-2541(93)90238-E.

Johnson, B.J., Fogel, M.L., and Miller, G.H. 1998. Stable isotopes in modern ostrich eggshell: a calibration for paleoenvironmental applications in semi-arid regions of southern Africa. Geochim. Cosmochim. Acta, **62**(14): 2451–2461. doi:10.1016/S0016-7037(98)00175-6.

Jolivet, A., Bardeau, J.-F., Fablet, R., Paulet, Y.-M., and de Pontual, H. 2008. Understanding otolith biomineralization processes: new insights into microscale spatial distributions of organic and mineral fractions from Raman microspectrometry. Anal. Bioanal. Chem. **392**(3): 551–560. doi:10.1007/s00216-008-2273-8. PMID:18665353.

Kalish, J.M. 1991. ^{13}C and ^{18}O isotopic disequilibria in fish otoliths: metabolic and kinetic effects. Mar. Ecol. Prog. Ser. **75**: 191–203. doi:10.3354/meps075191.

Karasov, W.H., and Martinez del Rio, C. 2007. Physiological ecology. Princeton University Press, Princeton, New Jersey.

Kennedy, B.P., Klaue, A., Blum, J.D., Folt, C.L., and Nislow, K.H. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Can. J. Fish. Aquat. Sci. **59**(6): 925–929. doi:10.1139/f02-070.

Klee, M.S. 1985. Modern practice of gas chromatography. Wiley, New York.

Mateo, I. 2009. Assessing essential fish habitat and connectivity of temperate and tropical fish populations using otolith microchemistry and stable isotopes. Ph.D. thesis, Department of Fisheries, Animal and Veterinary Sciences, University of Rhode Island, Kingston, Rhode Island.

McCarthy, M.D., Benner, R., Lee, C., Hedges, J.I., and Fogel, M.L. 2004. Amino acid carbon isotopic fractionation patterns in oceanic dissolved matter: an unaltered photoautotrophic source for dissolved organic nitrogen in the ocean? Mar. Chem. **92**(1–4): 123–134. doi:10.1016/j.marchem.2004.06.021.

McMahon, K.W. 2011. Functional connectivity of coral reef fishes in a tropical seascape assessed by compound-specific sable isotope analyses. Ph.D. thesis, Biological Oceanography, Massachusetts Institute of Technology and Woods Hole Oceanographic Institution Joint Program in Oceanography/Applied Ocean Science and Engineering, Woods Hole, Massachusetts.

McMahon, K.W., Fogel, M.L., Elsdon, T.S., and Thorrold, S.R. 2010. Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. J. Anim. Ecol. **79**(5): 1132–1141. doi:10.1111/j.1365-2656.2010.01722.x. PMID:20629794.

Morales-Nin, B. 1986. Structure and composition of otoliths of Cape hake *Merluccius capensis*. S. Afr. J. Mar. Sci. **4**(1): 3–10. doi:10.2989/025776186784461639.

Morales-Nin, B. 2000. Review of the growth regulation processes of otolith daily increment formation. Fish. Res. **46**(1–3): 53–67. doi:10.1016/S0165-7836(00)00133-8.

Mulcahy, S.A., Killingley, J.S., Phleger, C.F., and Berger, W.H. 1979. Isotopic composition of otoliths from a benthopelagic fish, *Coryphaenoides acrolepis*, Macrouridae: Gadiformes. Oceanol. Acta, **2**: 423–427.

Murayama, E., Takagi, Y., Ohira, T., Davis, J.G., Greene, M.I., and Nagasawa, H. 2002. Fish otolith contains a unique structural protein, otolin-1. Eur. J. Biochem. **269**(2): 688–696. doi:10.1046/j.0014-2956.2001.02701.x. PMID:11856329.

Murayama, E., Takagi, Y., and Nagasawa, H. 2004. Immunohistochemical localization of two otolith matrix proteins in the otolith and inner ear of the rainbow trout, *Oncorhynchus mykiss*: comparative aspects between the adult inner ear and embryonic otocysts. Histochem. Cell Biol. **121**(2): 155–166. doi:10.1007/s00418-003-0605-5. PMID:14689310.

O'Donnell, T.H., Macko, S.A., Chou, J., and Wehmiller, J.F. 2007. Stable carbon isotope composition of amino acids in modern and fossil *Mercenaria*. Org. Geochem. **38**(3): 485–498. doi:10.1016/j.orggeochem.2006.06.010.

Ostermann, D.R., and Curry, W.B. 2000. Calibration of stable isotope data: an enriched $\delta^{18}O$ standard used for source gas mixing detection and correction. Paleoceanography, **15**(3): 353–360. doi:10.1029/1999PA000411.

Popp, B.N., Graham, B.S., Olson, R.J., Hannides, C.C.S., Lott, M.J., López-Ibarra, G.A., Galván-Magaña, F., and Fry, B. 2007. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. *In* Stable isotopes as indicators of ecological change. *Edited by* T.D. Dawson and R.T.W. Siegwolf. Elsevier/Academic Press, Amsterdam, the Netherlands. pp. 173–190.

Post, D. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology, **83**(3): 703–718. doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2.

Reeds, P.J. 2000. Dispensable and indispensable amino acids for humans. J. Nutr. **130**(7): 1835S–1840S. PMID:10867060.

Rieley, G. 1994. Derivatization of organic compounds prior to gas chromatographic–combustion–isotope ratio mass spectrometry analysis: identification of isotope fraction processes. Analyst (Lond.), **119**(5): 915–919. doi:10.1039/an9941900915.

Rubenstein, D.R., and Hobson, K.A. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends Ecol. Evol. (Amst.), **19**(5): 256–263. doi:10.1016/j.tree.2004.03.017. PMID:16701265.

Sasagawa, T., and Mugiya, Y. 1996. Biochemical properties of water-soluble otolith proteins and the immunobiochemical detection of the proteins in serum and various tissues in Tilapia *Oreochromis niloticus*. Fish. Sci. **62**: 970–976.

Schwarcz, H.P. 1991. Some theoretical aspects of isotope paleodiet studies. J. Archaeol. Sci. **18**(3): 261–275. doi:10.1016/0305-4403(91)90065-W.

Schwarcz, H.P., Gao, Y., Campana, S., Browne, D., Knyf, M., and Brand, U. 1998. Stable carbon isotope variations in otoliths of Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **55**(8): 1798–1806. doi:10.1139/f98-053.

Secor, D.H., Henderson-Arzapalo, A., and Piccoli, P.M. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? J. Exp. Mar. Biol. Ecol. **192**(1): 15–33. doi:10.1016/0022-0981(95)00054-U.

Silfer, J.A., Engel, M.H., Macko, S.A., and Jumeau, E.J. 1991. Stable carbon isotope analysis of amino-acid enantiomers by conventional isotope ratio mass spectrometry and combined gas-chromatography isotope ratio mass-spectrometry. Anal. Chem. **63**(4): 370–374. doi:10.1021/ac00004a014.

Silfer, J.A., Qian, Y., Macko, S.A., and Engel, M.H. 1994. Stable carbon isotope compositions of individual amino acid enantiomers in mollusk shell by GC/C/IRMS. Org. Geochem. **21**(6–7): 603–609. doi:10.1016/0146-6380(94)90006-X.

Solomon, C.T., Weber, P.K., Cech, J.J., Jr., Ingram, B.L., Conrad, M.E., Machavaram, M.V., Pogodina, A.R., and Franklin, R.L. 2006. Experimental determination of the sources of otolith carbon and associated isotopic fractionation. Can. J. Fish. Aquat. Sci. **63**(1): 79–89. doi:10.1139/f05-200.

Stephenson, P.C., Edmonds, J.S., Moran, M.J., and Caputi, N. 2001. Analysis of stable isotope ratios to investigate stock structure of red emperor and Rankin cod in northern Western Australia. J. Fish Biol. **58**(1): 126–144. doi:10.1111/j.1095-8649.2001.tb00503.x.

Thorrold, S.R., Campana, S.E., Jones, C.M., and Swart, P.K. 1997. Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish. Geochim. Cosmochim. Acta, **61**(14): 2909–2919. doi:10.1016/S0016-7037(97)00141-5.

Thorrold, S.R., Latkoczy, C., Swart, P.K., and Jones, C.M. 2001. Natal homing in a marine fish metapopulation. Science (Washington, D.C.), **291**(5502): 297–299. doi:10.1126/science.291.5502.297. PMID:11209078.

Tieszen, L.L., and Fagre, T. 1993. Effect of diet quality and composition on the isotopic composition of respiratory CO_2, bone collagen, bioapatite, and soft tissues. *In* Molecular archaeology of prehistoric human bone. *Edited by* J. Lambert and G. Grupe. Springer-Verlag, Berlin, Germany. pp. 123–135.

Tohse, H., and Mugiya, Y. 2004. Sources of carbonate in fish otoliths: incorporation from bicarbonate and glucose. *In* Biomineralization: formation, diversity, evolution and application. *Edited by* I. Kobayashi and H. Ozawa. Tokai University Press, Tokyo, Japan. pp. 190–193.

Uhle, M.E., Macko, S.A., Spero, S.A., Engel, M.H., and Lea, D.W.

1997. Sources of carbon and nitrogen in modern planktonic foraminifera: the role of algal symbionts as determined by bulk and compound specific stable isotope analyses. Org. Geochem. **27**(3–4): 103–113. doi:10.1016/S0146-6380(97)00075-2.

Weidman, C.R., and Millner, R. 2000. High-resolution stable isotope records from North Atlantic cod. Fish. Res. **46**(1–3): 327–342. doi:10.1016/S0165-7836(00)00157-0.

West, J.B., Bowen, G.J., Dawson, T.E., and Tu, K.P. 2010. Isoscapes: understanding movement, pattern, and process on Earth through isotope mapping. Springer, New York.

Down, up, down and “smolting” twice? Seasonal movement patterns by juvenile steelhead (*Oncorhynchus mykiss*) in a coastal watershed with a bar closing estuary

Sean A. Hayes, Morgan H. Bond, Chad V. Hanson, Andrew W. Jones, Arnold J. Ammann, Jeffrey A. Harding, Alison L. Collins, Jeffrey Perez, and R. Bruce MacFarlane

Abstract: We investigated emigration behavior, habitat use, and Na$^+$,K$^+$-ATPase activity levels of juvenile steelhead trout (sea-run rainbow trout; *Oncorhynchus mykiss*) in Scott Creek, a small coastal watershed with a seasonally closed estuary in central California, USA. Migratory fish moved downstream in the spring with elevated Na$^+$,K$^+$-ATPase activity levels. Larger fish (>150 mm) moved downstream during February and March, leaving the stream, whereas fish moving between April and June were typically smaller and tended to recruit to the estuary. The Na$^+$,K$^+$-ATPase activity levels of estuarine fish varied during the summer as a function of salinity and temperature, but overall levels declined from peak spring values. Many summer recruits were observed retreating upstream into the watershed when estuarine water quality declined in the fall. Rather than entering the ocean when winter storms reconnected the estuary with the ocean in early winter, many migrated downstream several months later during subsequent springs. The largest smolts observed (>190 mm fork length (L_F)) were primarily those that had reared in the estuary the previous summer. Smolts were observed making a single migration from the upper watershed, but they were smaller (~120–190 mm L_F) with potentially reduced marine survival. In summary, we observed fish moving between freshwater and estuarine habitats seasonally and adjusting their osmoregulatory physiology as needed.

Résumé : Nous avons étudié le comportement d’émigration, l’utilisation de l’habitat et les niveaux d’activité de la Na$^+$,K$^+$-ATPase chez de jeunes truites-arc-en-ciel anadromes (*Oncorhynchus mykiss*) dans Scott Creek, un petit bassin versant côtier avec un estuaire qui est fermé une partie de l’année dans le centre de la Californie, É.-U. Les poissons migrateurs se déplacent au printemps avec des niveaux élevés d’activité de la Na$^+$,K$^+$-ATPase. Les poissons plus grands (>150 mm) se déplacent vers l’aval en février et mars et quittent le cours d’eau, alors que les poissons qui se déplacent entre avril et juin sont typiquement plus petits et tendent à se rassembler dans l’estuaire. Les niveaux d’activité de la Na$^+$,K$^+$-ATPase des poissons dans l’estuaire varient au cours de l’été en fonction de la salinité et de la température, mais diminuent en général à partir des valeurs maximales du printemps. On a observé plusieurs des recrues d’été retourner en amont dans le bassin versant lorsque la qualité de l’eau de l’estuaire diminue en automne. Au lieu de pénétrer dans l’océan lorsque les tempêtes hivernales rattachent à nouveau l’estuaire à l’océan au début de l’hiver, de nombreux poissons migrent vers l’aval plusieurs mois plus tard durant les printemps suivants. Les saumoneaux observés les plus gros (>190 mm longueur à la fourche (L_F)) sont en majorité ceux qui se sont développés dans l’estuaire durant l’été précédent. On observe des saumoneaux faire une migration unique à partir du bassin versant supérieur, mais ce sont des poissons plus petits (~120–190 mm L_F) dont la survie en mer est potentiellement réduite. En résumé, nous avons observé des poissons qui se déplacent aux diverses saisons entre les habitats d’eau douce et de l’estuaire et qui ajustent leur physiologie d’osmorégulation selon leurs besoins.

[Traduit par la Rédaction]

Received 9 September 2010. Accepted 24 March 2011. Published at www.nrcresearchpress.com/cjfas on 27 July 2011.
J22007

Paper handled by Associate Editor Michael Bradford.

S.A. Hayes, M.H. Bond[*], C.V. Hanson[†], A.W. Jones[‡], A.J. Ammann, J.A. Harding, A.L. Collins[§], J. Perez, and R.B. MacFarlane. Fisheries Ecology Division, NOAA National Marine Fisheries Service, Southwest Fisheries Science Center, 110 Shaffer Road, Santa Cruz, CA 95060, USA.

Corresponding author: S.A. Hayes (e-mail: sean.hayes@noaa.gov).

[*] Present address: University of Washington, School of Aquatic and Fishery Sciences, Box 355020, Seattle, WA 98195, USA.
[†] Present address: Department of Forest Science, 321 Richardson Hall, Oregon State University, Corvallis, OR 97331, USA.
[‡] Present address: Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, USA.
[§] Present address: Department of Forest Sciences, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada.

Can. J. Fish. Aquat. Sci. **68**: 1341–1350 (2011) doi:10.1139/F2011-062 Published by NRC Research Press

Introduction

Pacific salmon (*Oncorhynchus* spp.) and steelhead trout (*Oncorhynchus mykiss*, sea-run rainbow trout, hereafter called steelhead) exhibit tremendous life history variation that is typically interpreted as an adaptive response to temporal or spatial variability (Shapovalov and Taft 1954; Hendry and Stearns 2004; Satterthwaite et al. 2009). For example, sockeye salmon (*Oncorhynchus nerka*) exhibit marked polymorphism in their preferred spawning habitat, and this association is thought to exert divergent selective pressures on adult morphology (Blair et al. 1993). Chinook salmon (*Oncorhynchus tshawytscha*), on the other hand, exhibit consistent temporal segregation among spawning runs, with as many as four runs returning to a single river (Fisher 1994). While, Pacific salmon are thought to show comparable levels of variation in earlier portions of their life cycle, until recently logistical challenges have limited the study of this variation.

Of particular interest, but often poorly understood, are the juvenile life history forms, which make use of estuarine habitat (Healey 1982). Estuaries are extremely heterogeneous ecosystems, varying spatially and temporally in their chemistry, physical parameters, and community composition. Thus, myriad trade-offs between opportunities for growth and the risk of predation are all likely to influence the use of this habitat by juvenile salmonids (Wallace and Collins 1997; Roby et al. 2003; Bottom et al. 2005). Because these trade-offs likely underlie adaptive differences within and among populations, they are of particular interest to both biologists and managers.

In coastal California, like many Mediterranean climate regions, streams often have seasonally closing estuaries that form small freshwater lagoons when summer sandbars form at the estuarine mouths (Largier and Taljaard 1991; Schwarz and Orme 2005). The migratory behavior of salmonids in these systems is dictated by the availability of this seasonal habitat. As the marine survival size threshold for steelhead is typically 150 mm (Ward and Slaney 1988; Bond et al. 2008), in California watersheds that lack lagoon habitat, steelhead migrants tend to spend more time rearing upstream and are both older (age 2+) and larger (>140 mm) when they migrate downstream to the ocean (Hanson 2008). In watersheds where lagoons form, juvenile steelhead typically migrate downstream in a broader range of sizes and ages, including many smaller and younger fish (mean fork length (L_F) ~ 100 mm), most of which have elevated Na^+,K^+-ATPase activity levels indicative of seawater readiness (Hayes et al. 2004, 2008; Hanson 2008). A proportion of these smaller juveniles (~20%) remain in fresh water long enough to be "trapped" by the seasonal closure of the estuary (Bond et al. 2008). These lagoon-reared fish experience very high growth rates for an additional 6 months before winter storms reconnect the stream with the ocean (Smith 1990; Hayes et al. 2008). This additional growth is thought to confer substantially increased survival to adulthood, with estuarine-reared fish producing approximately 60%–90% of the returning anadromous adult steelhead in several California streams (Shapovalov and Taft 1954; Bond et al. 2008; Atkinson 2010). It is commonly assumed that these fish simply enter the ocean once winter storms increase stream flow enough to break through the sand bar. However, Shapovalov and Taft (1954) observed large numbers of juvenile steelhead migrating upstream during the late fall in Waddell Creek, presumably from the lagoon. The authors suspected that these fish subsequently re-emigrated downstream again, but lacked the equipment to track their fate and ultimately test their suspicion.

Here we sought to develop a more comprehensive understanding of both the physiological and behavioral adaptations that accommodate such a plastic life history in steelhead. Specifically, we investigated the relationships among patterns of movement, habitat quality, and physiological state by combining data from repeated captures, tagging, and a physiological assay for seawater readiness to better understand the seasonal movement into and out of the estuary–lagoon habitat in Scott Creek. This is of particular interest because previous studies suggest that emigrants have decoupled the migratory behaviors and physiological changes associated with smoltification from actual ocean entry (Hayes et al. 2004; Hanson 2008). This is quite unusual for salmonids (Clarke and Hirano 1995; Quinn et al. 2000) and therefore of particular interest. In addition, the timing of when these lagoon-reared fish actually enter the ocean needs clarification, as the potential for a reverse upstream migration may demand connectivity between upper watersheds and estuaries during the driest time of year. Therefore, a better understanding of the physiological ecology of estuarine-reared fish may be critical to management and conservation for these imperiled steelhead populations at the southern end of their geographic range and has implications for undocumented life history strategies in northern stocks. This is especially true given that estuaries and other coastal zones are often heavily impacted by anthropogenic stressors such as development and pollution, and connectivity with upper watersheds is often lost because of dewatering practices.

Materials and methods

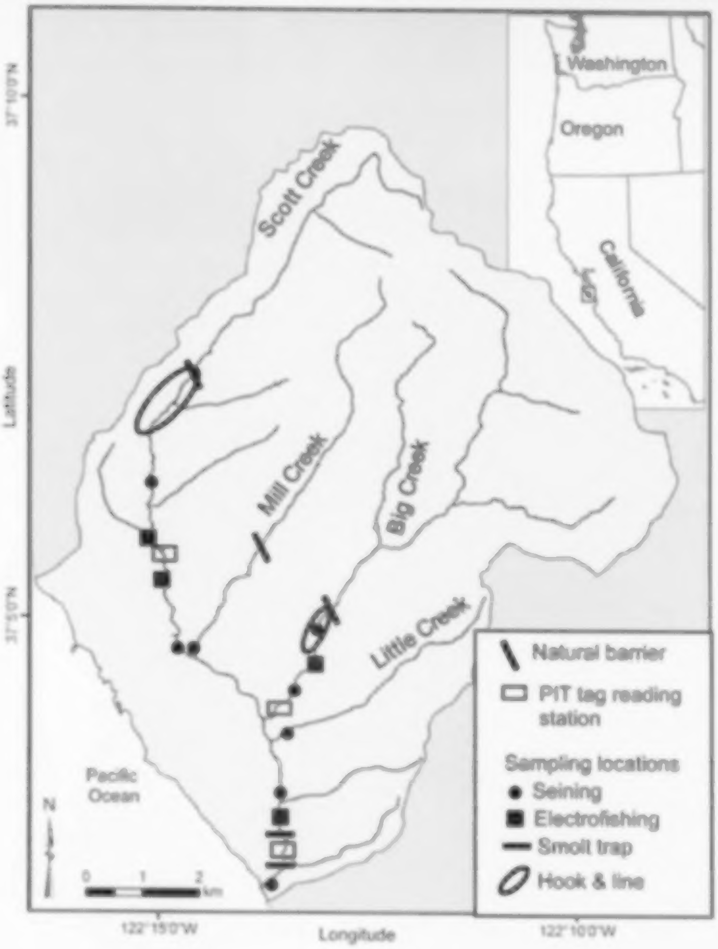
Study site

Scott Creek is a small coastal watershed, approximately 70 km^2 in area and 100 km south of San Francisco, California, USA (37°02′N). Anadromous fish can access approximately 23 km of stream between the estuary and natural upstream barriers of the main stem and the three main tributaries: Big Creek, Little Creek, and Mill Creek (Fig. 1). A small estuary is present, which typically becomes a freshwater lagoon during summer and fall when a sandbar forms at the creek mouth, blocking access to the ocean. A small conservation hatchery operates in the watershed and releases smolt-stage steelhead each spring. These fish are all marked by adipose fin clip and were excluded from this study.

Environmental measurements

Water temperature in the estuary was measured hourly using IB-Cod temperature loggers (Alpha Mach, Mont St-Hilaire, Quebec) from May 2002 to June 2003. These were replaced by two YSI 600 XLM data loggers (YSI Inc., Yellow Springs, Ohio), which recorded salinity, dissolved oxygen, and pH, from July 2003 to the present. To capture the maximum variation in parameters, one recorder was positioned on the bottom and the other floated just below the surface.

Fig. 1. Map of Scott Creek watershed (California, USA) showing locations where different sampling methods were used.


Washington
Oregon
California
Scott Creek
Mill Creek
Big Creek
Little Creek
Pacific Ocean
N
0 1 2 km
Natural barrier
PIT tag reading station
Sampling locations
● Seining
■ Electrofishing
Smolt trap
Hook & line
37°10'0"N
Latitude
37°5'0"N
122°15'0"W
Longitude
122°10'0"W

Fish sampling

Throughout the study fish were collected from three sites: in the upper watershed, at smolt traps on the main stem of Scott Creek, and in the estuary. These sites were sampled consistently between March 2002 and June 2008 (Fig. 1). Because of the physical differences of the sites and the availability of sampling equipment, multiple sampling methods were used. Fish in the upper watershed were sampled by seine or hook and line from May 2002 through December 2008. Beginning in 2006, electrofishing surveys were conducted three times per year in four 100 m stream reaches in the upper watershed. Smolt trapping was conducted from 2002 to 2005 by means of a two-chambered square mesh smolt trap with soft mesh wing walls extending to each bank. The trap was operated 3 days per week throughout the year except during exceptionally high flows associated with winter storms. From 2006 to 2008, the trap design was upgraded to a fixed-panel-and-pipe trap with a two-chamber live box and fished 7 days per week from January through July, flow permitting. All mesh spacing was ≤0.635 cm. Fish in the estuary (downstream of the smolt trap) were captured using a 30 m × 2 m beach seine with 0.95 cm square mesh wings and a 0.635 cm square mesh bag.

Fish were handled according to the methods of Hayes et al. (2004). Briefly, all fish were measured for L_F (mm) and mass (g). Gill samples (about five to six filaments) were collected from approximately 10 fish >100 mm L_F from each of the three locations each month through December 2004 to measure Na^+,K^+-ATPase activity. Most fish > 65 mm L_F collected after March 2003 received a passive integrated transponder (PIT) tag (11.5 mm FDX-B Glass Transponder, Allflex, Boulder, Colorado) by intraperitoneal injection with a 12 gauge needle. Fish were then placed in a recovery container for at least 10 min before release. As described in Hayes et al. (2008), growth rates were calculated for all recaptured PIT-tagged fish based on the change in measured fork length between capture events.

Na^+,K^+-ATPase

We used McCormick's (1993) nonlethal method of sampling to minimize mortality of these fish, which are listed in the Endangered Species Act, to measure activity levels of the enzyme Na^+,K^+-ATPase (hereafter referred to as ATPase activity) as a proxy for assessing seawater readiness (Björnsson and Bradley 2007). Gill samples were put into individual vials containing SEI buffer (150 mmol sucrose·L^{-1}, 10 mmol EDTA·L^{-1}, 50 mmol imidazole·L^{-1}, pH 7.3) and placed on dry ice in the field, followed by storage at –80 °C until laboratory analysis within 3 months of sample collection. Specific ATPase activity was expressed as micromoles of ADP per milligram protein per hour (μmol ADP·mg protein^{-1}·h^{-1}) in accordance with McCormick's (1993) nonlethal micro method. The homogenate of each sample was assayed in triplicate to evaluate within-sample variance.

Fish movement

Fish movements were assessed through a combination of instream PIT tag readers (Bond et al. 2007) and opportunistic recaptures. An instream PIT tag reader was located in the lower portion of the watershed at the head of the estuary and began operation in April 2004. Two additional readers were installed in December 2005: one on Big Creek and one on the Scott Creek main stem above the confluence with Mill Creek (Fig. 1).

Data analysis

Trapping effort and efficiency varied among years because of variable weather and flow conditions, staffing, and equipment resources and performance. As a result, downstream migrant capture rates were pooled across years and binned by month, with the total number of fish caught per month divided by the number of trapping days that occurred in that month across the course of the study to provide an estimate of the number of downstream migrants captured per daily trapping effort in a given month.

Owing to variable PIT tag reader detection efficiencies, as well as periods when readers and (or) traps were inoperable, all reported detection rates represent minimum estimates. For similar reasons, data were pooled across years and binned by month or day of year unless otherwise specified, and interannual variation was not evaluated because of the confounding effects of inconsistent and improving sampling methods.

We investigated the influence of size and timing of migration through the smolt trap on lagoon recruitment. This was done by assessing the number of fish in a given year that were PIT-tagged when captured at the trap and subsequently detected in the basin after lagoon closure, either by a PIT tag reader or by recapture in the estuary. Fish that were not resighted were assumed to have entered the ocean or died, although it is likely that some fish recruited but were not resighted. Fish that were tagged after lagoon closure in a given year were excluded from analysis.

Results

Downstream migration trends

Fish movement peaked in the spring, typically between the months of March and June. There was a general trend of larger fish being caught between December and March (with a dip in January) and smaller smolts captured during the rest of the year (Supplemental Fig. S1[1]). Mean L_F for all downstream migrants measured during the course of the study was 110 mm (standard deviation (SD) ± 39 mm, $n = 3848$). Overall there is a long tail in the length frequency of juvenile steelhead downstream migrants in this system and a wide range of size classes (Fig. 2*a*).

ATPase activity dynamics

A total of 695 gill samples were collected between March 2002 and December 2004 and successfully measured in the laboratory for ATPase activity, with coefficients of variation (CV) of <20% between replicate wells. ATPase activity levels were log-transformed to achieve normality and analyzed with a linear mixed model that included year as a random variable. ATPase activity levels differed significantly between locations ($F_{[2,681]} = 22.19$, $p < 0.001$) and at the seasonal time scale ($F_{[3,681]} = 21.095$, $p < 0.001$). No interaction was observed between season and location ($F_{[6,681]} = 0.50$, $p = 0.806$). Pairwise comparisons by location and season that proved significant are reported in Supplemental Table S1[1]. Seasonal patterns of ATPase activity at the smolt trap and in the estuary are shown (Fig. 2*b*), with data binned by month regardless of year collected.

Fish sampled in the estuary during spring typically had elevated ATPase activity (Fig. 2*b*; Supplemental Table S1[1]). ATPase activity was typically lower during the period of estuary closure (summer–fall), but it varied among years. As environmental variables were likely to influence this, multiple regression was used to compare ATPase activity of fish on the day they were sampled in the estuary with the 2-week running average of salinity and temperature leading up to that date. A significant effect was observed ($F_{[2,77]} = 41.121$, $p < 0.001$, adjusted multiple $R^2 = 0.516$), with salinity (standard coefficient (SC) = 0.672, $p < 0.001$) appearing to have a greater effect than temperature (SC = –0.279, $p = 0.001$) for the range of environmental conditions observed. Specifically, higher ATPase levels were associated with higher salinities, while lower ATPase levels were associated with higher temperatures.

Estuary recruitment trends

Timing of migration strongly influenced the probability of recruitment to the estuary (Fig. 2*c*), with later-migrating fish having a higher probability of recruiting to the lagoon ($t_{[345]} = 16.8$, $p < 0.0001$). A comparison of the size frequency distributions for initial tagging at the smolt trap of resighted fish with that of fish that were not resighted revealed a lack of larger fish recruiting to the estuary (Fig. 2*a*, Kolmogorov–Smirnov two-sample $p = 0.032$, $n = 2296$).

ATPase activity levels were measured for 107 fish that were PIT-tagged prior to estuary closure in 2003 and 2004. A comparison of fish that subsequently recruited to the estuary ($n = 15$) with fish that were not resighted ($n = 92$) found that fish that were confirmed recruiting to the estuary tended to have lower ATPase activity, were significantly shorter in L_F, and migrated later in the year (Table 1).

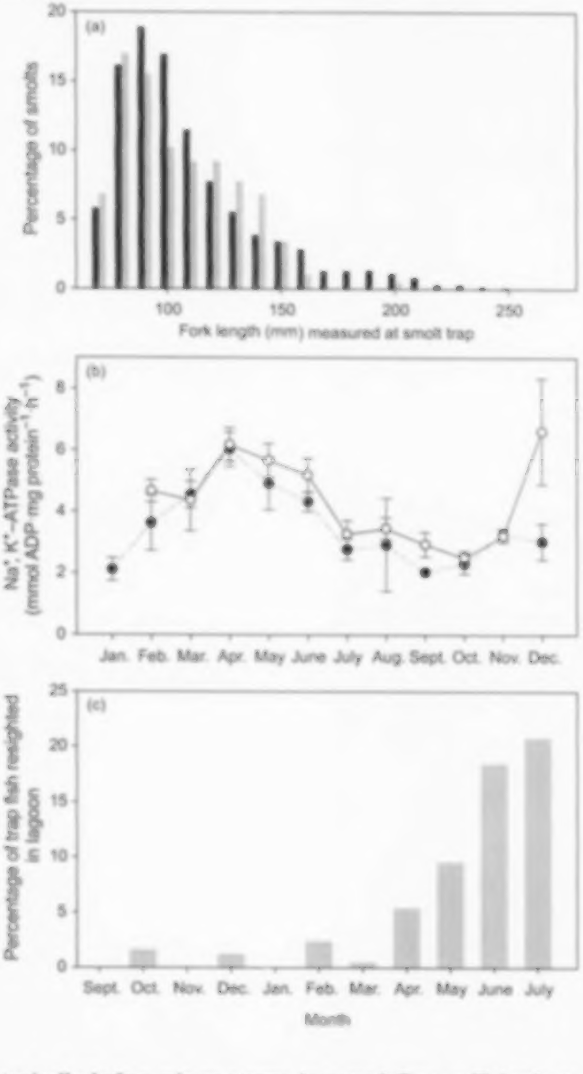
Post-tagging movement in freshwater (Do some fish smolt twice?)

Many estuary fish moved upstream from the lagoon in fall,

---

[1] Supplementary data are available with the article through the journal Web site (http://www.nrcresearchpress.com/cjfas).

Fig. 2. Seasonal dynamics of early and late downstream migrants in a comparison of (*a*) size distributions of fish tagged at the smolt trap that either were (gray bars) or were not (black bars) resighted in the estuary after sandbar formation between 2003 and 2008; (*b*) Na^+,K^+-ATPase activity levels averaged by month (all years combined, ±1 standard error, SE) for fish sampled at the smolt trap (solid circles) and the estuary (open circles) in the lower watershed; and (*c*) the percentage of fish tagged at the smolt trap on a monthly basis that were later resighted in the estuary–lagoon after sandbar formation.



typically before winter storms increased flow sufficiently to breach the sandbar. During the course of this study (2002–2008), the mean bar breach date was 7 December, with a range of 14 November to 20 December. Of the fish tagged or resighted in the lagoon after bar closure each year (n = 1236), 45% were detected upstream at some point and 28% were detected upstream after bar breach. Upstream PIT tag reader detections of estuary fish peaked in November for the reader a short way above the estuary, often several weeks before sandbar breach. Detections peaked for the two readers in the upper watershed in January (Fig. 3*a*).

This upstream movement coincided with deteriorating water quality conditions in the estuary as fall progressed. To reduce noise in temperature and salinity measurements, the rolling 2-week average of each day from July 2003 to December 2008 was calculated and then averaged again by day of year across years. Lagoon salinity increased during fall, as salt water was pushed over the sandbar by large swells from storms in the North Pacific moving down the coast. Dissolved oxygen subsequently dropped, potentially caused by die-off of freshwater algae in response to increasing salinity. Note that while the moving average for dissolved oxygen of the lower sonde hovers around 5 $mg \cdot L^{-1}$, there are many daily averages during mid-October through November between 0 and 1 $mg \cdot L^{-1}$, before continuing to decline in dissolved oxygen and increase in salinity during December (Figs. 3*b* and 3*c*).

Many of the tagged fish detected migrating upstream spent several additional months in the upper watershed and were captured a second time at the smolt trap during the following spring. A comparison of recaptures at the smolt trap between fish initially tagged in the upper watershed versus the estuary during the previous season shows that downstream movement of estuary fish through the smolt trap peaked in March, while upper watershed fish movement peaked in April ($t_{[146]} = 4.8$, $p < 0.0001$, Fig. 4*a*) and that the estuary fish were significantly larger ($t_{[147]} = 12.7$, $p < 0.0001$, Fig. 4*b*). However, winter growth rates in the upper watershed for the two groups were not significantly different, with estuary fish having a slightly higher absolute growth rate of 0.22 ± 0.11 $mm \cdot day^{-1}$ (mean ± SD, n = 69) than upstream fish (0.19 ± 0.11 $mm \cdot day^{-1}$, n = 94). In addition, as estuary fish were typically longer to begin with, their specific growth rates (percent change in $L_f \cdot day^{-1}$) were lower (0.13 ± 0.08) than fish reared solely upstream (0.16 ± 0.19).

Estuary-reared fish captured at the smolt trap each spring are probably the source of almost all smolts greater than 190 mm in Scott Creek (Fig. 4*b*). The relative contribution of tagged fish detected at the smolt trap from the estuary versus those on their first migration from the upper watershed is difficult to determine because of varying trap and PIT tag reader detection efficiencies. However, roughly 4% of all upstream-tagged fish were recaptured at the smolt trap. In comparison, 6% of all estuary-tagged fish from the previous year were recaptured at the trap or detected by PIT tag readers the following year, suggesting the estuary is making a major contribution to the subsequent spring's smolt run each year, particularly in the larger size classes (especially if one adjusts for annual trap efficiencies, which ranged from ~10% to 50%; S.A. Hayes, unpublished data).

ATPase activity appears to differ between fish on the "first" and "second" migration. Unfortunately, the ATPase activity data were collected prior (2002–2004) to acquisition of PIT-tag-based movement data, so the exact life history pathway of larger ATPase-sampled fish moving through the smolt trap is not documented for that time. However, when comparing ATPase activity of fish above and below the 150 mm L_f marine survival size threshold (Bond et al. 2008), it appears that larger fish, which are more likely to enter the ocean after leaving the smolt trap, have higher ATPase

Table 1. Mean values ± SD comparing several variables between downstream migrants sampled for Na$^+$,K$^+$-ATPase activity (μmol ADP·mg protein^{-1}·h^{-1}) that recruited to the estuary for the summer versus fish that were not resighted.

	Estuary recruit	Not resighted	*t* score	*p* (two-tail)
Na$^+$,K$^+$-ATPase activity	3.07±2.09	4.09±2.25	2.09	0.097
Fork length (mm)	130.20±38.08	153.47±41.63	2.09	0.037
Timing of migration (day of year)	149.87±39.26	103.52±51.89	2.07	<0.001

Fig. 3. Evidence that steelhead that recruit to the lagoon during summer months migrate back upstream in fall based on (*a*) monthly detections of estuary fish by instream PIT tag readers located upstream of the estuary in the upper (black bars) and lower watershed (gray bars — detections are limited to one detection per individual fish per day). Further evidence includes declining estuary water quality conditions for two sondes, one floating at the surface (gray line) and one mounted on the bottom (black line), based upon 2-week rolling averages of (*b*) daily salinity and (*c*) dissolved oxygen for the period of July 2003 through December 2008. Data are average by day of year across years to display general trends.

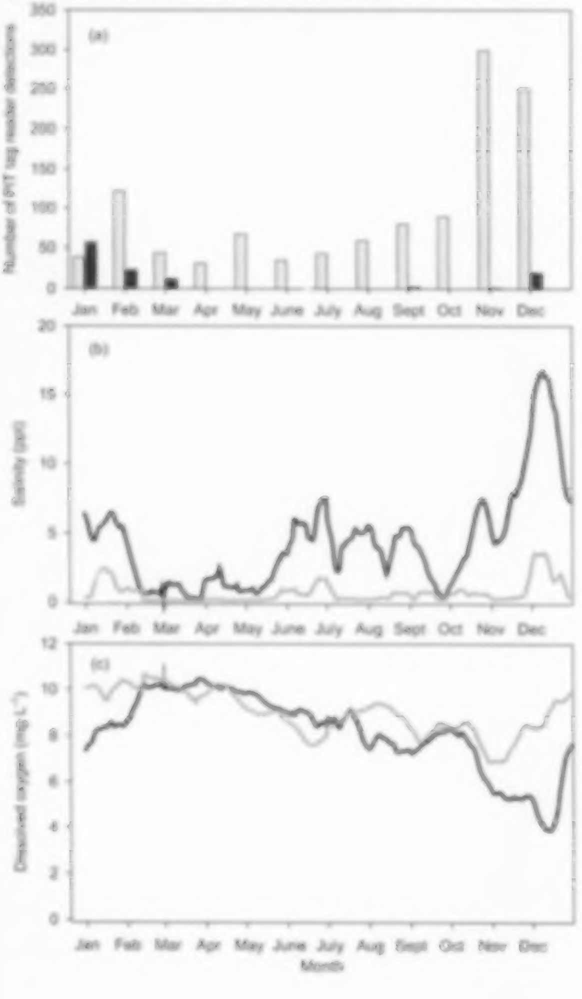
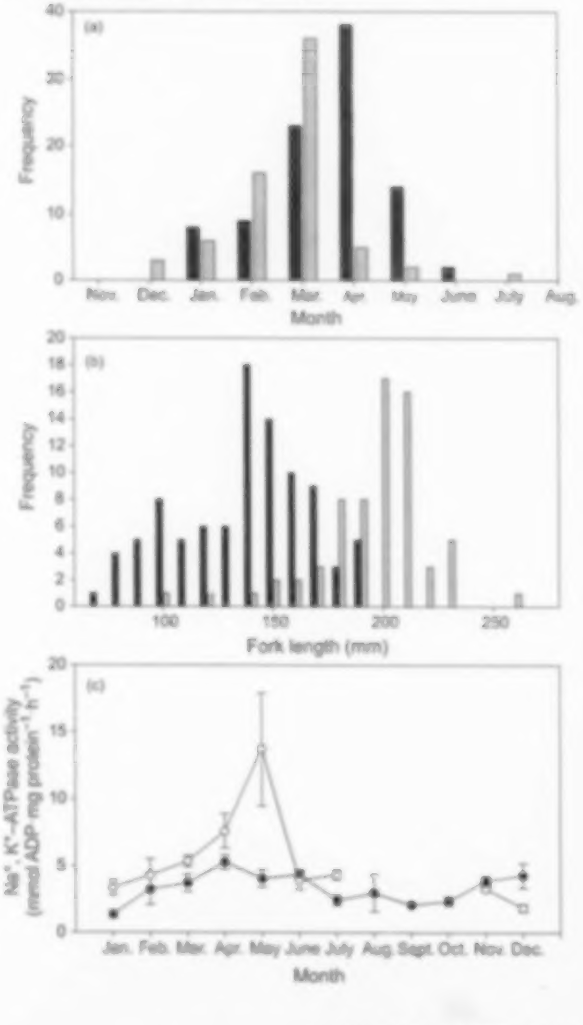


Fig. 4. (*a*) Fish that migrate upstream from the estuary in fall make a second downstream migration during the spring smolt outmigration period (gray bars), earlier than fish on their first migration (black bars) based on a comparison of the timing of downstream migration through the smolt trap or passive integrated transponder (PIT) tag reader by fish last seen or tagged in either upstream or estuarine habitat (during previous year). (*b*) Fish on their second migration are larger based on a comparison of the size frequency distribution of fish recaptured at the smolt trap that were last seen or tagged in either upstream (black bars) or estuarine habitat (gray bars). (*c*) Associated with that are the Na$^+$,K$^+$-ATPase activity levels at the smolt trap for fish <150 mm (solid circles) or ≥150 mm (open circles) marine survival size threshold, indicating larger fish are more prepared for seawater entry.



levels (Fig. 4c; $t_{[1,30]} = 1.98$, $p = 0.001$) during the months of January through May. As 11 of the 37 fish in the larger size class were >190 mm L_{F}, it is likely that many of these fish were on their second migration and about to enter the ocean.

Discussion

The results of this study help to illustrate the extent of life history plasticity steelhead are capable of exhibiting to make use of available habitat. Downstream-migrating fish in Scott Creek demonstrate a wide range of size classes and degree of smoltification as indicated by Na^+,K^+-ATPase activity levels. Larger fish (>150 mm) moved downstream during February and March and exited the stream, whereas fish moving during April through June were smaller and tended to recruit to the estuary. Many summer estuary recruits were observed retreating upstream into the watershed when estuarine water quality declined each fall and remained there until performing a second downstream migration several months later during subsequent spring, representing the largest smolts in the system. The observation of regular upstream migration by juvenile steelhead after entering the estuary but prior to ocean entry is rather unusual, with important ecological and evolutionary implications and important consequences for management.

Initial downstream migration and estuary habitat use

Larger fish migrated downstream earlier, typically in February and March, whereas smaller fish were observed migrating in April through June (Supplemental Fig. S1[1]). These data are concordant with historical records from the neighboring watershed, Waddell Creek (Shapovalov and Taft 1954). It is now apparent that the larger fish are likely heading directly to sea, whereas the smaller fish are recruiting to the estuary. Corresponding differences in ATPase levels were observed in the present study, with the larger, earlier-moving fish having more elevated ATPase activity. A concurrent laboratory experiment conducted with fish from Scott Creek observed a positive relationship between length and probability of surviving a seawater challenge, indicating that larger fish from this stock have greater osmoregulatory capacity (Beakes et al. 2010).

Water temperatures at the smolt trap are typically between 7 and 13 °C during February and March, with daily highs reaching 17 °C by June. Warm temperatures have been shown to inhibit ATPase expression (Adams et al. 1975), with steelhead typically requiring temperatures < 13 °C to smolt (McCullough 1999; Myrick and Cech 2004). It is likely that smaller size and warmer temperatures contributed to the lower ATPase values observed in the later-migrating fish. However, the ATPase values of these later migrants were still elevated compared with upper watershed nonmigratory fish and suggest an increased level of seawater readiness. In a comparative study of coastal watersheds in central California with and without estuary–lagoon habitat, Hanson (2008) observed a lack of elevated ATPase activity in steelhead juveniles < 150 mm L_{F} from watersheds without estuaries, demonstrating a population level plasticity in the smoltification process that adjusts to individual watershed habitats.

Elevated ATPase levels observed in the late-migrating smaller fish suggests a bet-hedging strategy for two reasons. The first is that the estuary–lagoon habitat is not always available, with lagoon formation delayed until late August or September during some years, leaving the fish with a stretch of shallow riffle habitat and limited cover. Few fish are observed recruiting to the estuary in late summer. While their fate is uncertain, it is possible one reason for elevated ATPase levels is they may go to sea when suitable estuarine habitat is not present. Alternatively, they may be consumed by predators before ocean entry (Hayes et al. 2008, 2011). The second reason for potential bet-hedging is occasional spring elevated salinity levels in the estuarine habitat when recruitment is occurring, creating a potential need for enhanced osmoregulatory ability. ATPase activity of fish in the estuary varied in response to environmental conditions, with a substantial drop in activity levels during summers with low salinity and warm temperatures. This is not surprising and has been observed in Atlantic salmon (*Salmo salar*) as well (McCormick et al. 1999).

In Atlantic salmon, the smolt life history pathway is size-related, with a threshold length required to begin the physiological transformation (McCormick et al. 1998). Steelhead in some regions probably have a similar size-based smolting threshold. However, the results of this study and that of Hanson (2008), who observed elevated ATPase levels in migrating fish as small as 70 mm L_{F}, indicate that steelhead can adapt to local habitat conditions and, under certain circumstances, decouple the minimum size threshold from elevated ATPase levels and migration behavior. Some readers may argue that these smaller fish were not true smolts. It is likely they were not, as the vast majority of downstream migrants in this study exhibited parr coloration and only began to silver after recruiting to the estuary (S.A. Hayes, personal observation), when ATPase levels are decreasing and growth is increasing (Hayes et al. 2008). It was not until these fish retreated upstream and made their second downstream migration that they showed additional signs of smoltification, including complete silvering, reduced condition factor, darkened fin tips, and the highest levels of ATPase activity. At the same time, some of the larger fish coming out of the upper watershed on their initial migration also demonstrated smolt characteristics, indicating that multiple life history pathways are present in this system. We conclude that steelhead have the plasticity to adjust behavioral and physiological processes typically associated with smoltification and spread these processes out in stages to take advantage of variable habitat availability and suitability. The decoupling of the smoltification process from actual ocean entry serves to further complicate the concept of a "smolt", which was recently described as "a physiological state which continues to defy definition" (Björnsson and Bradley 2007).

Upstream migration and "smolting" twice

Historical studies from the Scott and Waddell creek watersheds during the 1930s and 1940s report almost identical size frequency distributions of downstream migrants as found in the present study, but concluded this was primarily the result of upstream rearing (Shapovalov and Taft 1954). The authors reported upstream movement of juvenile fish in Waddell Creek and suggested that there were probably growth advantages in the estuarine habitat, speculating on the processes described in our study. Shapovalov and Taft (1954) also lament their inability to track the movements of individual fish through the steps of initial downstream migration, estuarine recruitment, fall upstream migration, and subsequent spring downstream migration. Upstream movement by juvenile salmonids in the parr stage is not uncommon, having been reported in both Atlantic salmon and coho salmon (*Oncorhynchus kisutch*) (Tschaplinski and Hartman 1983; Hutchings 1986; Koski 2009). What is unusual about the data reported here is that this upstream migration takes place after the parr–smolt transformation has begun. Upstream movement may simply be necessary to avoid poor habitat conditions in lagoons. While there are occasional saltwater intrusions during summer, there are typically low salinity levels and extensive freshwater algal production. Storm surges deposit seawater over the sandbar each fall, and the lagoon habitat generally becomes anoxic when freshwater algae dies and reduces oxygen levels in the estuary as it biodegrades. More frequent saltwater intrusions in fall and early winter may make the estuary unsuited to the physiological state of juveniles.

The life history strategy and physiology of "smolting twice" does not appear to have been previously documented in a wild population of salmonids. Atlantic salmon held under laboratory conditions have demonstrated the ability to regress when prevented from migrating and then go through the smoltification process a second time 1 year later (Shrimpton et al. 2000). From a physiological perspective, it is not surprising for an iteroparous anadromous salmonid to undergo this process more than one time. The phenomenon may increase in frequency as one moves south through the steelhead range, where some stocks in southern California may not have access to the ocean for 1 or more years because of dry conditions. In a simple sense, these steelhead have adapted to take advantage of optimal rearing habitat, somewhat like sockeye salmon or Atlantic salmon using lakes for rearing habitat (Hutchings 1986; Quinn 2005). Because of limited watershed size and location of this rearing habitat, they have developed a "circular" migration pattern, moving down and up in the watershed to make maximal use of available habitat. In a recent review, Koski (2009) identifies that coho salmon "nomads", a life history type previously thought of as displaced fry and parr that do not survive, are actually exhibiting similar movement behaviors in many populations across their range.

What remains a mystery is why estuary fish remain in the upper watershed for so long after upstream migration. Most fish are well over the 150 mm marine survival threshold, and the longest distance they could travel is only about 10 km in this watershed, which they could traverse in a night or two. Yet many delay their actual ocean entry for 2–3 months after the onset of winter storms and the reconnection of the stream with the ocean. Growth was moderate during this time, and the added size may outweigh mortality risks during those months. In addition, with the primary cue for smoltification being photoperiod (Björnsson and Bradley 2007), it may simply be a mechanistic timing issue for these fish to complete the "resmoltification" process. Alternatively, this may be indicative of some critical time window for seawater entry to begin their ocean migration. Little is known about steelhead ocean migratory behavior, other than that they have the largest smolt size requirements of any Pacific salmonid (Ward et al. 1989; Groot and Margolis 1991; Bond et al. 2008), are rarely observed in the California Current ecosystem, and are typically found only in a band along the 50°N parallel (Welch et al. 1998). All of this suggests a major ocean migration that may be coupled in a critical way to synchronizing movements with optimal current patterns. Recent work on the Scott Creek population using archival tags identified a very narrow ocean thermal habitat window and suggests that central California steelhead have the same migratory destination in the North Pacific as more northern populations and are likely to feed in the California Current while transiting north, timing their outmigration from central California to narrow temperature windows between February and April (Hayes et al. 2011).

Our results emphasize the importance of protecting estuarine habitat for steelhead rearing in coastal watersheds. The largest smolts migrating downstream each spring in Scott Creek had previously reared in the estuary and were not products of upstream habitats alone. Despite the intact and arguably high quality of stream habitat within Scott Creek, most of its steelhead production comes from juveniles using the estuary for considerable growth, highlighting the fact that the rearing capacity of these coastal watersheds is limited even under the best of circumstances. Finally, this study highlights the critical need to provide adequate flow in what is a fairly dry Mediterranean climate to maintain connectivity between lagoon and upstream habitats. It is likely that even the most naturally functioning coastal lagoons have seasons when the lagoon habitat becomes degraded. In such cases it is necessary to maintain an exit corridor for steelhead to retreat upstream, away from deteriorating water quality.

Acknowledgements

Funding for this study was provided by the National Marine Fisheries Service, the California Central Coast Salmon Restoration Program, and the California Fisheries Restoration Grant Program. Field assistance was provided by M. Atcheson, M. Beakes, S. Campbell, M. Durighello, L. Schilpp, D. Gottesman, E. Freund, K. Hauser, T. Hollenbeck, D. Kaufman, J. Krupa, C. Phillis, C. Michel, I. Ralston, D. Schperberg, S. Sogard, D. Swank, E. Sturm, R. Weidling, and J. Wong. Landowner access was provided by Big Creek Lumber Company, the Rowley family, the Wilson family, and Cal Poly's Swanton Pacific Ranch. Animal studies were approved by the UCSC Animal Use Committee and carried out according to National Institutes of Health guidelines and National Marine Fisheries Service Endangered Species Act Section 10 permit No. 1112.

References

Adams, B.L., Zaugg, W.S., and McLain, L.R. 1975. Inhibition of salt water survival and Na-K-ATPase elevation in steelhead trout (*Salmo gairdneri*) by moderate water temperatures. Trans. Am. Fish. Soc. **104**(4): 766–769. doi:10.1577/1548-8659(1975)104<766:ioswsa>2.0.co;2.

Atkinson, K.A. 2010. Adundance and growth of steelhead (*Oncorhynchus mykiss*) in a California lagoon. M.Sc. thesis, San Jose State University, San Jose, Calif.

Beakes, M.P., Satterthwaite, W.H., Collins, E., Swank, D., Merz, J.E., Titus, R., Sogard, S., and Mangel, M. 2010. Smolt transformation in two California steelhead populations: effects of temporal variability in growth. Trans. Am. Fish. Soc. **139**(5): 1263–1275. doi:10.1577/T09-146.1.

Björnsson, B.T., and Bradley, T.M. 2007. Epilogue: Past successes, present misconceptions and future milestones in salmon smoltification research. Aquaculture, **273**(2–3): 384–391. doi:10.1016/j.aquaculture.2007.10.020.

Blair, G.R., Rogers, D.E., and Quinn, T.P. 1993. Variation in life history characteristics and morphology of sockeye salmon in the Kvichak River System, Bristol Bay, Alaska. Trans. Am. Fish. Soc. **122**(4): 550–559. doi:10.1577/1548-8659(1993)122<0550:VILHCA>2.3.CO;2.

Bond, M.H., Hanson, C.V., Baertsch, R., Hayes, S.A., and MacFarlane, R.B. 2007. A new low cost in-stream antenna system for tracking passive integrated transponder (PIT) tagged fish in small streams. Trans. Am. Fish. Soc. **136**(3): 562–566. doi:10.1577/T06-084.1.

Bond, M.H., Hayes, S.A., Hanson, C.V., and MacFarlane, R.B. 2008. Marine survival of steelhead (*Oncorhynchus mykiss*) enhanced by a seasonally closed estuary. Can. J. Fish. Aquat. Sci. **65**(10): 2242–2252. doi:10.1139/F08-131.

Bottom, D.L., Jones, K.K., Cornwell, T.J., Gray, A., and Simenstad, C.A. 2005. Patterns of Chinook salmon migration and residency in the Salmon River estuary (Oregon). Estuar. Coast. Shelf Sci. **64**(1): 79–93. doi:10.1016/j.ecss.2005.02.008.

Clarke, W.C., and Hirano, T. 1995. Osmoregulation. *In* Physiological ecology of Pacific salmon. *Edited by* C. Groot, L. Margolis, and W.C. Clarke. UBC Press, Vancouver, B.C. pp. 319–377.

Fisher, F.W. 1994. Past and present status of Central Valley chinook salmon. Conserv. Biol. **8**(3): 870–873. doi:10.1046/j.1523-1739.1994.08030863-5.x.

Groot, C., and Margolis, L. 1991. Pacific salmon life histories. UBC Press, Vancouver, B.C.

Hanson, C.V. 2008. Influence of lagoon habitat on steelhead life history variation. M.A. thesis, University of California, Santa Cruz, Santa Cruz, Calif.

Hayes, S.A., Bond, M.H., Hanson, C.V., and MacFarlane, R.B. 2004. Interactions between endangered wild and hatchery salmonids; Can the pitfalls of artificial propagation be avoided in small coastal streams? J. Fish Biol. **65**(Suppl. A): 101–121. doi:10.1111/j.0022-1112.2004.00547.x.

Hayes, S.A., Bond, M.H., Hanson, C.V., Freund, E.V., Smith, J.J., Anderson, E.C., Ammann, A., and MacFarlane, R.B. 2008. Steelhead growth in a small central California watershed: upstream and estuarine rearing patterns. Trans. Am. Fish. Soc. **137**(1): 114–128. doi:10.1577/T07-043.1.

Hayes, S.A., Bond, M.H., Wells, B.K., Hanson, C.V., Jones, A.W., and MacFarlane, R.B. 2011. Using archival tags to infer habitat use of Central California steelhead and coho salmon. *In* Advances in tagging and marking technologies for fisheries management and research. *Edited by* B. Parsons, J. McKenzie, M. Mesa, Q. Phelps, A. Seitz, J. Pepperell, and R.K. Kopf. American Fisheries Society, Auckland, N.Z. [In press.]

Healey, M.C. 1982. Juvenile Pacific salmon in estuaries: the life support system. *In* Estuarine comparisons. *Edited by* V.S. Kennedy. Academic Press, New York. pp. 315–341.

Hendry, A.P., and Stearns, S.C. 2004. Evolution illuminated: salmon and their relatives. Oxford University Press, New York.

Hutchings, J.A. 1986. Lakeward migrations by juvenile Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. **43**(4): 732–741. doi:10.1139/f86-090.

Koski, K.V. 2009. The fate of coho salmon nomads: the story of an estuarine-rearing strategy promoting resilience. Ecol. Soc. **14**(1): 4. Available from http://www.ecologyandsociety.org/vol14/iss1/art4/.

Largier, J.L., and Taljaard, S. 1991. The dynamics of tidal intrusion, retention, and removal of seawater in a bar-built estuary. Estuar. Coast. Shelf Sci. **33**(4): 325–338. doi:10.1016/0272-7714(91)90061-F.

McCormick, S.D. 1993. Methods for nonlethal gill biopsy and measurement of Na^+, K^+ -ATPase activity. Can. J. Fish. Aquat. Sci. **50**(3): 656–658. doi:10.1139/f93-075.

McCormick, S.D., Hansen, L.P., Quinn, T.P., and Saunders, R.L. 1998. Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **55**(Suppl. 1): 77–92. doi:10.1139/d98-011.

McCormick, S.D., Cunjak, R.A., Dempson, B., O'Dea, M.F., and Carey, J.B. 1999. Temperature-related loss of smolt characteristics in Atlantic salmon (*Salmo salar*) in the wild. Can. J. Fish. Aquat. Sci. **56**(9): 1649–1658. doi:10.1139/f99-099.

McCullough, D.A. 1999. A review and synthesis of the effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to chinook salmon. US Environmental Protection Agency, Portland, Ore. EPA 910-R-99-010.

Myrick, C.A., and Cech, J.J. 2004. Temperature effects on juvenile anadromous salmonids in California's central valley: What don't we know? Rev. Fish Biol. Fish. **14**: 113–123. doi:10.1007/s11160-004-2739-5.

Quinn, T.J. 2005. The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle, Wash.

Quinn, T.P., Unwin, M.J., and Kinnison, M.T. 2000. Evolution of temporal isolation in the wild: genetic divergence in timing of migration and breeding by introduced chinook salmon populations. Evolution, **54**(4): 1372–1385. PMID:11005303.

Roby, D.D., Lyons, D.E., Craig, D.P., Collis, K., and Visser, G.H. 2003. Quantifying the effect of predators on endangered species using a bioenergetics approach: Caspian terns and juvenile salmonids in the Columbia River estuary. Can. J. Zool. **81**(2): 250–265. doi:10.1139/z02-242.

Satterthwaite, W.H., Beakes, M.P., Collins, E.M., Swank, D.R., Merz, J.E., Titus, R.G., Sogard, S.M., and Mangel, M. 2009. Steelhead life history on California's central coast: insights from a state-dependent model. Trans. Am. Fish. Soc. **138**(3): 532–548. doi:10.1577/T08-164.1.

Schwarz, K.M., and Orme, A.R. 2005. Opening and closure of a seasonal river mouth: The Malibu Estuary–Barrier–Lagoon system, California. Zeitschrift Fur Geomorphologie Supplementband, **141**: 91–109.

Shapovalov, L., and Taft, A.C. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*). Calif. Fish Game Fish Bull. 98.

Shrimpton, J.M., Bjornsson, B.T., and McCormick, S.D. 2000. Can Atlantic salmon smolt twice? Endocrine and biochemical changes during smolting. Can. J. Fish. Aquat. Sci. **57**(10): 1969–1976. doi:10.1139/f00-143.

Smith, J.J. 1990. The effects of sandbar formation and inflows on aquatic habitat and fish utilization in Pescadero, San Gregorio, Waddell and Pomponio Creek Estuary/Lagoon Systems, 1985–1989. Interagency Agreement 84-04-324, San Jose State University, prepared for California Department of Parks and Recreation. Available from http://cdm15025.contentdm.oclc.org/cgi-bin/showfile.exe?CISOROOT=/p267501ccp2&CISOPTR=149&filename=150.pdf.

Tschaplinski, P.J., and Hartman, G.F. 1983. Winter distribution of juvenile coho salmon (*Oncorhynchus kisutch*) before and after logging in Carnation Creek, British Columbia, and some implication for overwinter survival. Can. J. Fish. Aquat. Sci. **40**(4): 452–461. doi:10.1139/f83-064.

Wallace, M., and Collins, B.W. 1997. Variation in use of the Klamath River Estuary by juvenile Chinook salmon. Calif. Fish Game, **83**(4): 132–143.

Ward, B.R., and Slaney, P.A. 1988. Life history and smolt-to-adult survival of Keogh River steelhead trout (*Salmo gairdneri*) and the relationship to smolt size. Can. J. Fish. Aquat. Sci. **45**(7): 1110–1122. doi:10.1139/f88-135.

Ward, B.R., Slaney, P.A., Facchin, A.R., and Land, R.W. 1989. Size-biased survival in steelhead trout (*Oncorhynchus mykiss*): back-calculated lengths from adults' scales compared to migrating smolts at the Keogh River, British Columbia. Can. J. Fish. Aquat. Sci. **46**(11): 1853–1858. doi:10.1139/f89-233.

Welch, D.W., Ishida, Y., Nagasawa, K., and Eveson, J.P. 1998. Thermal limits on the ocean distribution of steelhead trout (*Oncorhynchus mykiss*). N. Pac. Anadr. Fish. Comm. Bull. 1. pp. 396–404.

Elemental fingerprints of southern calamary (*Sepioteuthis australis*) reveal local recruitment sources and allow assessment of the importance of closed areas

Gretta T. Pecl, Sean R. Tracey, Leonid Danyushevsky, Simon Wotherspoon, and Natalie A. Moltschaniwskyj

Abstract: Movement of individuals over a range of temporal and spatial scales is a critical process in determining the structure and size of populations. For most marine species, a substantial amount of movement that is responsible for connecting subpopulations occurs when individuals are too small and numerous to be tagged using conventional methods. Using the elemental fingerprints of the statoliths of the squid *Sepioteuthis australis* and a robust machine learning classification technique, this study determined that newly hatched squid had elemental signatures that exhibited sufficient spatial variation to act as natural tags for natal origin and that elemental signatures can be used to allocate adult squid back to their natal site. Between 55% and 84% of the adult squid caught throughout the east and southeast of Tasmania, Australia, were classified back to an area that is closed to commercial fishing over much of the peak spawning period, and this was the only location with substantive evidence of natal recruitment. Although many studies have demonstrated the potential of this approach to discern connectivity between population units, few studies have successfully done so by then examining the trace element profiles of adults in addition to those of hatchlings as we have demonstrated with *S. australis*.

Résumé : Les déplacements des individus sur une gamme d'échelles temporelles et spatiales sont des éléments essentiels dans la détermination de la structure et de la taille des populations. Pour la plupart des espèces marines, une partie majeurs des déplacements qui sont responsables des liens entre les sous-populations se produit lorsque les individus sont trop petits et nombreux pour être marqués à l'aide des méthodes conventionnelles. En utilisant les empreintes en éléments des statolithes du calmar *Sepioteuthis australis* et une technique robuste de classification par apprentissage automatique, notre étude démontre que les calmars nouvellement éclos possèdent une signature d'éléments qui montre suffisamment de variation spatiale pour servir d'étiquette naturelle afin de déterminer leur origine à la naissance; les signatures d'éléments peuvent aussi servir à associer les calmars adultes à leur site de naissance. Entre 55 % et 84 % des calmars adultes capturés sur l'ensemble de l'est et du sud-est de la Tasmanie, Australie, ont été classifiés comme provenant d'une région fermée à la pêche commerciale durant une grande partie de la période maximale de fraie; c'est la seule région qui présente des preuves solides de recrutement natal. Bien que plusieurs études aient démontré le potentiel de cette approche pour discerner la connectivité entre les unités démographiques, peu de travaux ont réussi à le faire en examinant les profils des éléments en trace des adultes en plus de ceux des nouveau-nés comme nous l'avons fait chez *S. australis*.

[Traduit par la Rédaction]

Introduction

Quantifying many of the processes that structure marine metapopulations is an enigmatic problem in ecology, although it is widely acknowledged that populations of marine species are connected on temporal and spatial scales larger than their local habitat through dispersal of juveniles and (or) the migration of adults. Movement of individuals among habitat patches is an essential component of both the maintenance and establishment of populations. The capacity of individuals to recolonize areas following localized extinction events, because of natural or human-mediated change, is highly dependent on the strength of connectivity among subpopulations. Given that migration affects persistence, coloni-

Received 13 May 2010. Accepted 26 March 2011. Published at www.nrcresearchpress.com/cjfas on 19 August 2011.
J21809

Paper handled by Associate Editor Bronwyn Gillanders.

G.T. Pecl and S.R. Tracey. Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49, Tasmania, Australia 7001.
L. Danyushevsky. Centre for Ore Deposit Research, University of Tasmania, Private Bag 79, Tasmania, Australia 7001.
S. Wotherspoon. School of Mathematics and Physics, University of Tasmania, Private Bag 37, Tasmania, Australia 7001.
N.A. Moltschaniwskyj. National Centre for Marine Conservation and Resource Sustainability, University of Tasmania, Locked Bag 1370, Tasmania, Australia 7250.

Corresponding author: Gretta T. Pecl (e-mail: Gretta.Pecl@utas.edu.au).

Can. J. Fish. Aquat. Sci. **68**: 1351–1360 (2011) doi:10.1139/F2011-059 **Published by NRC Research Press**

zation, and extinction, we need to explicitly measure and quantify migration rates among spawning populations. The source of new individuals, either via recruitment or migration, is usually unknown, and although the sum of the influx is measureable, quantifying the relative contribution of new individuals from a number of different sources is extremely problematic. In particular, tracking or marking large quantities of larvae or juveniles is often challenging, and yet this is frequently a life history stage where large-scale movements occur (Thorrold et al. 2007).

The successful use of spatial and temporal control measures to manage exploitation of wild populations requires knowledge of the likely sources of recruitment to local populations (i.e., is the population reproductively self-sustaining or dependent on immigration for the replacement of individuals; Holmes et al. 2003). This is necessary for sustainable management, including the placement and expected function of marine reserves (Cowen and Sponaugle 2009) or correct placement and timing of areas closed to commercial fishing to protect spawning individuals (Pecl et al. 2006; Fogarty and Botsford 2007). Without knowledge of the real population structure, the assumption in fisheries management that population units are homogeneous risks overfishing of less productive stocks (Begg et al. 1999), potentially affecting the long-term stability and sustainability of the entire stock (Hilborn et al. 2003). The level of genetic exchange arising from immigration between populations will also influence their long-term stability given naturally occurring levels of stochasticity in survival and growth (Havenhand 1995).

The integration of naturally occurring trace element signatures into the perennial biomineralized hard structures of fish and invertebrates provide ideal natural tags because of metabolic inertness of the structures and the chronological incorporation of elements that are influenced by environmental variables (Campana 1999; Gillanders 2005; Doubleday et al. 2008). Natural tags are suited to answering ecological questions regarding life histories, as all individuals regardless of size or life history stage are essentially tagged (Elsdon and Gillanders 2005), all individuals potentially represent recaptures, and it is possible to detect small-scale movement (Gillanders and Kingsford 2000). Trace element tags in the hard structures of a wide range of taxa have been used to distinguish between groups, including gastropods (Zacherl et al. 2003), squid (Ikeda et al. 2002; Warner et al. 2009), octopus (Doubleday et al. 2008), mussels (Becker et al. 2007), and fish (Gillanders et al. 2001). More rarely, studies have used trace element profiles of structures formed during the embryo phase to identify the geographic locations where the hard part was formed, thereby allowing the reconstruction of the natal source of adults from across a range of locations (Campana 1999; Becker et al. 2007). The overwhelming advantage of trace element analysis over genetic approaches is that it allows direct determination of natal origins of a large number of adults and can provide a direct measure of exchange over a discrete and defined period (Becker et al. 2007).

Squid are important components of many marine ecosystems from the poles to the equator, as their mid-level position in the food web means that they have important roles as both predators and prey. Worldwide, catches of cephalopods have increased rapidly over recent decades (Hunsicker et al. 2010), and unique aspects of their growth and reproduction mean that they are likely to play an increasingly important role in the oceans that are changing because of climate change (Pecl and Jackson 2008). Functionally, cephalopods are ecological equivalents of teleost fish except that they grow faster and have shorter life spans, and therefore they achieve much faster turnover of generations. Given the annual life span of many squid species, new "recruits" generally constitute the entire population for any one year and are solely responsible for producing the future population. The occurrence of spatially and temporally predictable spawning events of many inshore squids means they are vulnerable to targeted fishing of the spawning aggregations, and the single generation structure of populations can result in susceptibility to overfishing.

Sepioteuthis australis is a relatively large (~1–4 kg) but short-lived (<1 year), multiple-spawning inshore squid (Pecl 2001). A low level of spawning for this species occurs all year round; however, in the Australian spring and summer adults aggregate over seagrass beds in large numbers to spawn in shallow waters (<15 m), laying benthic strands attached to the substrate with 5–7 large eggs (6–8 mm) inside each strand, with multiple strands forming discrete egg masses (Moltschaniwskyj et al. 2003). Following escalating catch and effort in Tasmania, Australia, the fishery has been managed via short-term spatial and temporal closures of the major spawning areas. These closures have been put in place in the east coast of Tasmania (Great Oyster Bay and Mercury Passage), as most of the spawning activity is concentrated within these regions (Moltschaniwskyj and Pecl 2003). However, survival of southern calamary hatchlings is size selective (Steer et al. 2003*a*), indicating that not all spawning activity contributes equally to the next generation. Until now, we have not been able to show a direct link between putative "most important" spawning areas and adult fished recruits.

In this study we use trace element profiles to explore the extent of self-seeding, the degree of connectivity among local populations, and the extent of dispersal of hatchlings in southern calamary (*S. australis*). The key objectives of this study were to determine whether (*i*) hatchling elemental signatures exhibit sufficient spatial variation over regional scales to act as tags for natal origin and (*ii*) if the elemental signatures of the hatchlings from one year can be used to allocate adults caught the following year to a natal location. As the life span of southern calamary is less than 1 year, there is no overlap of generations, minimizing any confounding temporal effects. Additionally, as hatchlings are taken straight from very late-stage embryos laid directly attached to seagrass, the exact natal location is known for all hatchling individuals. This is in contrast with most studies examining natal origins in fish where only the broad region of origin is estimated for free-swimming larvae, reducing the potential to determine small-scale differences.

Materials and methods

Sample collection

A total of 218 southern calamary hatchlings were collected across six regions on Tasmania's east and southeast coast: Great Oyster Bay (GOB), Mercury Passage (MP), Frederick Henry Bay (FHB), Bruny Island (BI), Tasman Peninsula (TP), and D'Entrecasteaux Channel (DEC) in December 2004 and January 2005 (Table 1; Fig. 1). Hatchlings were collected in

Table 1. Location and date of collection of hatchling and adult *Sepioteuthis australis* used for statolith element analysis.

					Mantle length (mm)	
Region	Date	Sex	Collected *n*	Analysed *n*	Mean	Range
Hatchlings						
BI	7 December 2004	—	30	25	—	—
FHB	9 December 2004	—	20	11	—	—
MP	14 December 2004	—	20	10	—	—
TP	9 December 2004		20	19		
DEC	17 December 2004	—	30	20	—	—
GOB	22 December 2004	—	60	54	—	—
GOB	18 January 2005	—	100	79	—	—
Adults						
GOB	August 2005	M	95	31	343	225–493
		F	76	33	282	224–340
DEC	September 2005	F	2	2	281	280–282
MP	August 2005	M	78	20	360	214–457
		F	54	17	278	196–346
	September 2005	M	88	26	395	269–492
		F	10	5	296	270–318
TP	August 2005	F	11	6	264	190–300
	September 2005	M	16	5	326	258–490
		F	20	7	290	240–325

Note: Great Oyster Bay (GOB), Mercury Passage (MP), Bruny Island (BI), Tasman Peninsula (TP), Frederick Henry Bay (FHB), and D'Entreacasteaux (DEC).

situ from very late stage egg masses (Moltschaniwskyj et al. 2003) located on shallow (<10 m) seagrass beds by SCUBA divers. Hatchlings were collected by placing a plastic bag over the egg mass and gently agitating the mass to stimulate the late stage embryos (stage 28–29; Steer et al. 2003*b*) to hatch. Hatchlings were examined and those that had completely absorbed their yolk sac were retained and stored in 95% ethanol until statolith extraction. Owing to the small size of hatchling statoliths (~160–375 μm), fine-gauge sterile hypodermic needles were used to remove each statolith from the cartilaginous case. Hatchling statoliths were prepared for mounting directly after extraction.

Male and female adult southern calamary were collected during August and September 2005 from four geographic regions on Tasmania's east and southeast coast: GOB, MP, TP, and DEC (Table 1; Fig. 1). The animals were caught over or in close proximity to seagrass beds using squid jigs on rod and reel. Adults were frozen soon after capture and thawed prior to dissection and statolith extraction. Statoliths from adult calamary were extracted using ceramic-tipped forceps to reduce the possibility of contaminating the samples. Once extracted, the statoliths were stored in sealed polycarbonate multiwell trays.

Statolith preparation

One statolith from each animal was randomly selected for analysis. Up to 30 hatchling statoliths were placed longitudinally (to expose as much surface area to grinding as possible) in a circular mould, and epoxy resin was poured over the statoliths to produce a 1 cm deep mount. Adult statoliths were ground individually from the lateral dome to expose the hatch check in the lateral plain. The plane of grinding was precisely controlled using the edge of a glass slide as described by Lipinski and Durholtz (1994). Adult statoliths were then mounted in epoxy resin blocks (10–30 statoliths per block) with the ground surface exposed for polishing.

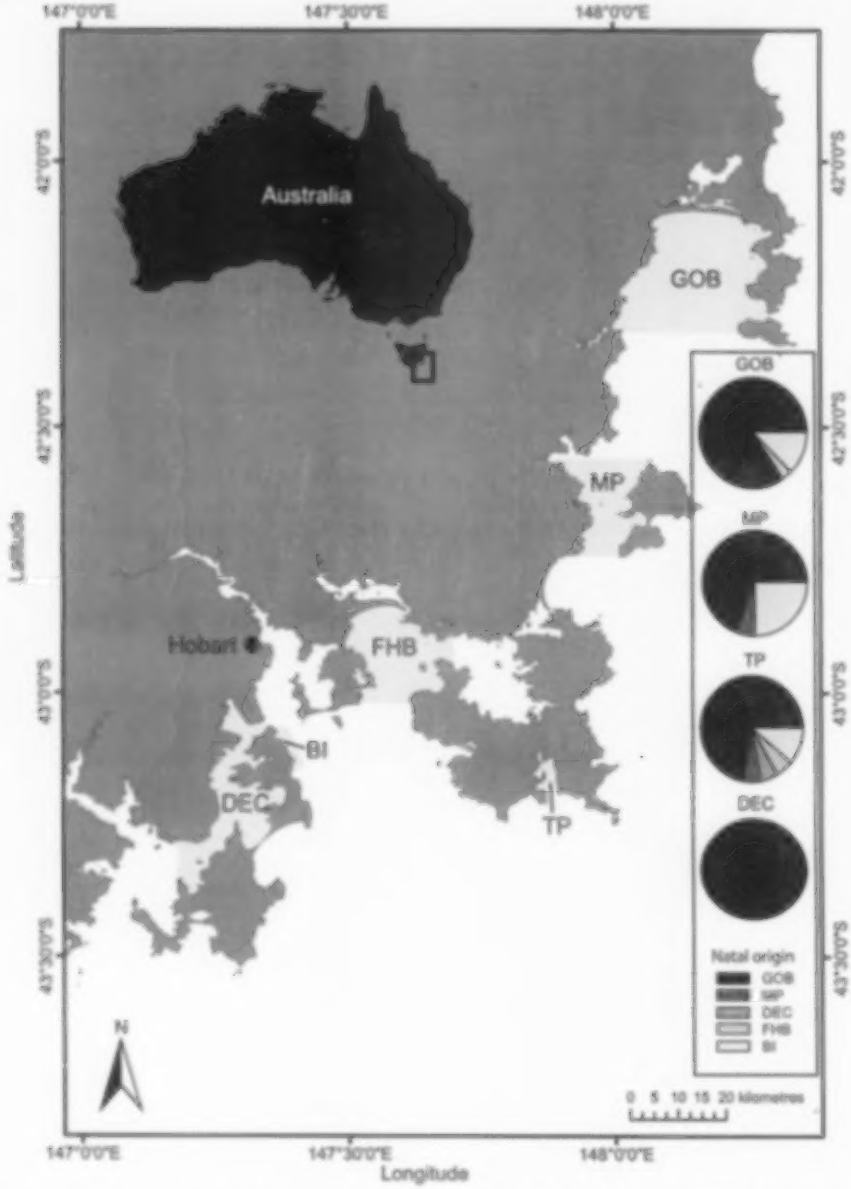
Once the epoxy blocks holding the statoliths were cured in an oven at approximately 40 °C, the statoliths in the blocks were ground using 1500 grit carborundum paper with 18.2 MΩ·cm^{-1} Milli-Q water then polished using 0.3 μm alumina powder on a suede polishing disc to reveal the inner ring structure in the dome of the statolith. Following polishing, the mounts were ultrasonically cleaned for 5 min in Milli-Q water and then rinsed further using Milli-Q water to remove surface contaminants before being allowed to air dry in a laminar flow fume hood.

Trace element analysis

Trace element analysis was conducted by laser ablation inductively coupled plasma – mass spectrometry (LA-ICP-MS) using a New Wave UP-213 Nd:YAG Q-switched laser ablation system controlled by the MEOLaser 213 software package, coupled with an Agilent HP4500 Quadrapole ICP-MS. The following suite of elements was recorded for each statolith: ^{7}Li, ^{24}Mg, ^{45}Sc, ^{47}Ti, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{85}Rb, ^{88}Sr, ^{137}Ba, and ^{43}Ca, with the latter used as an internal standard.

Ablations were conducted in "spot mode", with the statolith stationary under the pulsating laser beam, resulting in a continuous deepening in the cylindrical hole during ablation. Ablations were carried out in an atmosphere of ultra-high-purity helium using a custom-designed small-volume (~3 cm^{3}) sample cell. This cell ensured rapid transport of the ablated material to the mass spectrometer, resulting in accurate documentation of chemical variations with depth. For statolith analysis, the beam diameter was set nominally at

Fig. 1. Southern calamary hatchlings were collected from six known spawning regions from southeast Tasmania: GOB, Great Oyster Bay; MP, Mercury Passage; FHB, Fredrick Henry Bay; TP, Tasman Peninsula; BI, Bruny Island; and DEC, D'Entrecasteaux Channel. Adult calamary were caught from GOB, MP, TP, and DEC. The figure inset shows the proportion of adults that were classified as originating from each spawning region.



55 µm with a pulse rate of 5 Hz. To avoid error introduced by surface contamination, each statolith was pre-ablated at 2 Hz for 2 s. The average laser energy output was ~13 J·cm^{-2}. Prior to each ablation, background levels were collected for 30 s, and the average of these measurements was subtracted from the following sample measurement to correct for background levels. The acquisition time for each sample ranged between 40 and 60 s.

Elemental concentrations were calibrated against the international standard NIST612 following the procedure of Longerich et al. (1996). To adjust for instrument drift over the day, the standard was ablated approximately each hour or when a new mount was inserted into the ablation chamber and drift corrected using linear interpolation. The concentration of calcium in the statoliths was assumed to be constant at 40.04 percent weight CaO, based on $CaCO_3$ stoichiometry. Detection limits for each element were estimated from three standard errors of the background signal. Analytical precision is dependent on the concentration level and varies from ~2% (1 standard error) at high concentrations to ~30%–50% at concentrations near the detection limits. Accuracy is difficult to estimate because of the lack of international secondary standards, but is believed to be >50% based on our experience with analysis of other materials.

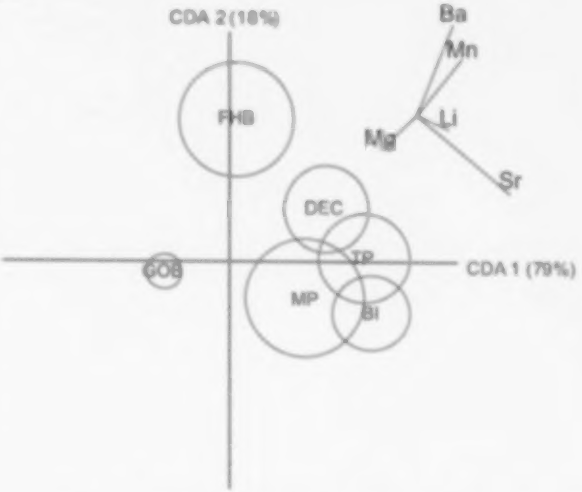
Hatchling statoliths were ablated in the centre of the dome region, which is the widest region of the statolith. No data was collected for 11% of the hatchling statoliths generally because the statoliths were too small for the laser beam width or they cracked during the ablation process. A much greater percentage (65%) of the data from the adult statoliths was rejected, as it was difficult to get a sufficient amount of ablation sample from the hatchling portion of the statolith, particularly if the statolith sections were too thin to allow sufficient time for data acquisition (Table 1). Adult statoliths were ablated in the middle of the hatch check, which is the dark region within the ring structure. An increase in the concentration of ^{55}Mn at the time of ablation was used as a diagnostic confirmation of successfully ablating within the prehatching zone (Brophy et al. 2004; Ruttenberg et al. 2005). Using ^{55}Mn as a diagnostic of the correct position of the ablation was useful if the statolith was not ground sufficiently and part of the posthatch statolith material was being ablated. Only the portion of the ablation signal where increased ^{55}Mn concentrations occurred was used for data analysis. There was greater than an order of magnitude difference between the concentration of ^{55}Mn in the statolith cores and the statolith material deposited after hatching.

Data analysis

Of the 15 elements recorded, only seven (Li, Mn, Mg, Fe, Sr, Ba, and the internal standard Ca) occurred consistently in the statoliths at concentrations greater than the detection limits. Concentrations of Fe were confounded between the analysis of the hatchling and the adult statoliths because of calibration issues. The remaining elements were used in the data analysis. All variables, within each regional group of hatchlings in the analyses, were checked for normality using quantile–quantile plots. Minor departures from normality were corrected by log transformation, and three outliers were removed. Spatial differences in the trace elements from the statoliths of hatching squid from the six regions were explored with a one-way multivariate analysis of variance (MANOVA), with a canonical discriminant analysis (CDA) then used to determine which regions differed and which of the trace elements could explain the pattern of differences. However, it should be noted that the allocation of hatchlings and adults to source regions was conducted with random forests (below). CDA and random forests operate via very different mechanisms. Insight gained from the discriminant procedures of CDA may not be consistent with the basis of sample allocation with the random forests analysis.

To characterize the trace element signatures of statoliths from hatchlings obtained from each of the six geographic regions, and to then classify the trace element signatures of adults caught from these locations in the following year, we used the random forests method of classification using Li, Mn, Mg, Sr, and Ba as predictor variables. Random forests (Breiman 2001) is a modern, computer intensive, ensemble classification technique that generalizes classification trees (Breiman et al. 1984). Random forests have quickly proven to be one of the most important algorithms in the machine learning literature, showing robust and improved results of classifications on standard data sets (Boinee et al. 2006). They also confer advantages in being resistant to overfitting and do not require the strict distributional assumptions required by classical discriminant methods (Breiman 2001; Izenman 2008).

Fig. 2. Canonical discriminate plot showing the variation in microchemistry of the hatchling statoliths from six regions along the east coast of Tasmania: Great Oyster Bay (GOB), Mercury Passage (MP), Bruny Island (BI), Tasman Peninsula (TP), D'Entrecasteaux Channel (DEC), Fredrick Henry Bay (FHB). Trace elements used in the analysis were Li, Mn, Mg, Sr, and Ba. Circles represent the 95% confidence ellipses around the centroid mean for each region.



Like classification trees, random forests generates a rule to classify observations into one of *K* predefined classes, given training data consisting of observations whose class is known. Random forests draws bootstrap samples from the training data and fits a classification tree (Breiman et al. 1984) to each bootstrap sample. New observations are classified by applying each of the fitted trees to the new observation and assigning the class selected by the majority of trees, with probabilities of class membership derived from the proportions of trees that select each class. In random forests, cross-validation or a separate test to get an unbiased estimate of the test set error are not necessary as the general classification accuracy can be judged from the "out-of-bag" (OOB) misclassification rate. Approximately two-thirds of the data in the training sample are extracted for each bootstrap sample, with the remaining one-third of the cases left as OOB data (Boinee et al. 2006). For each tree, the misclassification rate is computed for the OOB training data excluded from the bootstrap sample used to fit the tree, and these are then pooled to give an overall measure of classification accuracy. In this way the OOB data are used to get a running unbiased estimate of the classification error as trees are added to the forest. We assessed which trace elements played a greater role in partitioning the data into defined classes with the Gini importance measure, which incorporates a (weighted) mean of the individual tree's improvement in the splitting criterion produced by each variable (Friedman 2001). Every

Fig. 3. Mean concentrations (± standard error) of Li, Mn, Mg, Sr, and Ba in the statoliths of hatchlings from six sites in the southeast of Tasmania: Great Oyster Bay (GOB), Mercury Passage (MP), Bruny Island (BI), Tasman Peninsula (TP), D'Entrecasteaux Channel (DC), Fredrick Henry Bay (FH).

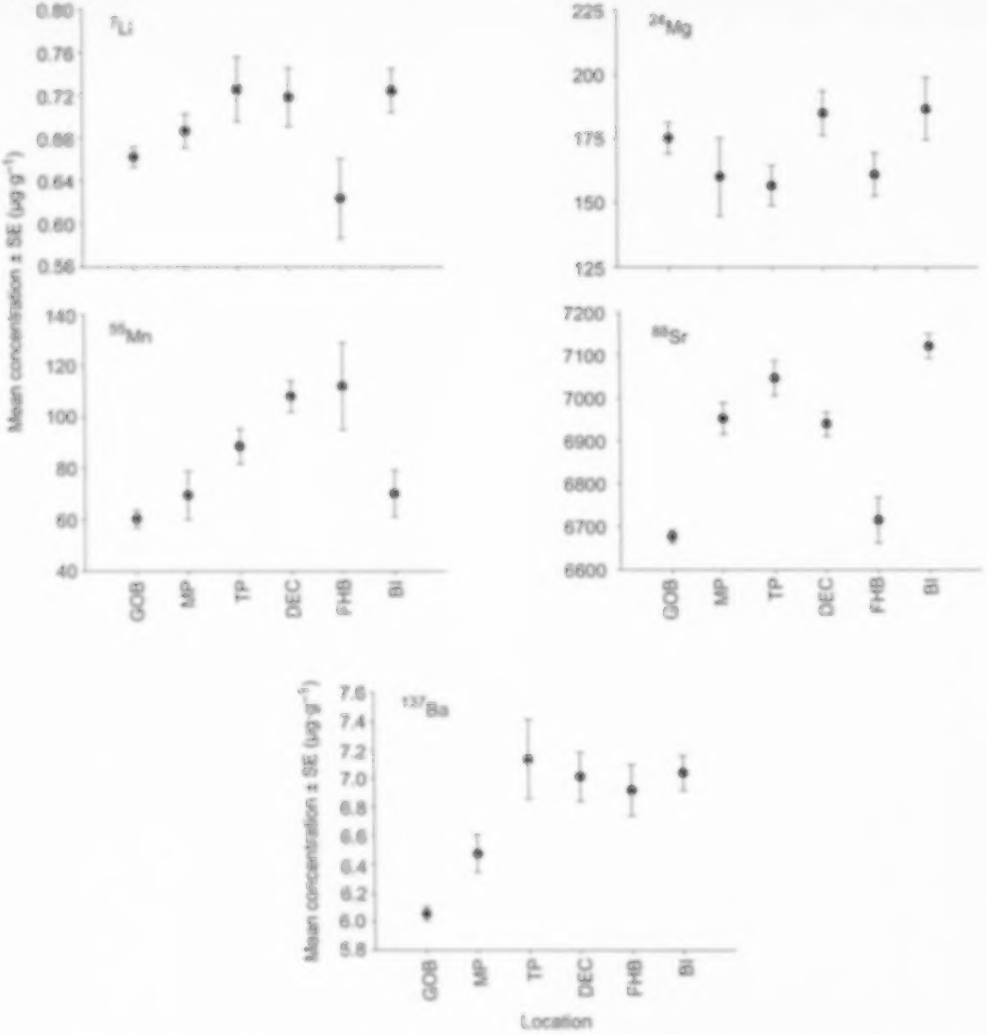
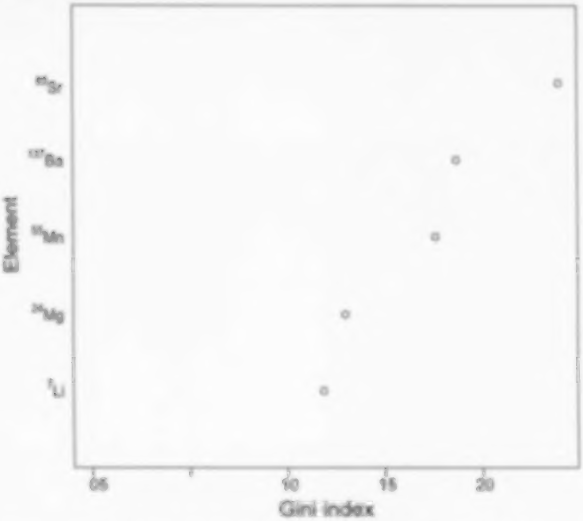


Table 2. Random forests accuracy of hatchlings allocated to each of the regions based on trace element profiles of the statolith.

			n						
Region	*N*	Accuracy (%)	GOB	MP	TP	DEC	FHB	BI	% misclassified to GOB
GOB	131	83.2	109	3	3	6	4	6	—
MP	10	20.0	1	2	1	2	0	4	10
TP	18	11.1	0	2	2	6	1	7	0
DEC	20	50.0	2	0	3	10	3	2	10
FHB	11	27.3	3	0	1	3	3	1	27
BI	25	60.0	2	2	5	1	0	15	8
Total	215	65.6							

Note: *N* is total number of hatchlings from each region; *n* is number of hatchlings classified to belong to each region.

Fig. 4. Variable importance in the random forest generation for classifying hatchlings to natal location, according to the Gini importance measure.



time a split of a node is made on a variable, the Gini impurity criterion for the two descendent nodes is less than the parent node. Adding up the Gini decreases for each individual variable over all trees in the forest gives a measure of variable importance.

Because of the nature of the sampling, samples from GOB were over-represented in the training data, and it is known that classifiers can perform poorly in such circumstances (Izenman 2008). To compensate, GOB was downsampled in the bootstrap, so that in each bootstrap sample only 20 individuals were drawn from GOB. Random forests were fitted using the random forests package (Liaw and Wiener 2002) in the R statistical computing environment (R Development Core Team 2010).

Results

Spatial variation in the elemental composition of hatchling statoliths

Significant differences were seen in the statolith elemental composition of squid hatched among the six spawning regions (F = 8.708; df = 25, 1045; $P < 0.001$). The statoliths of the hatchlings from GOB and FHB had distinctly different elemental compositions from all other regions (Fig. 2). GOB hatchlings had statoliths with relatively greater concentrations of Mg, but lower concentrations of Ba and Mn, while FHB hatchlings had statoliths with relatively lower concentrations of Sr and Li (Figs. 2 and 3). In contrast, hatchlings from DEC, MP, TP, and BI had statoliths with greater concentrations of Sr than hatchlings from other regions (Fig. 2).

Allocation of hatchlings and adults to regions based on statolith elemental composition

Generalized error was stabilized from 1000 trees; therefore, for high robustness of a forest and stable importance values we generated a forest of 5000 trees. The overall forest accuracy for hatchlings from the six regions, using Ba, Li, Mg, Mn, and Sr as classifiers, was 65.6% (Table 2). Hatchlings from MP, TP, and FHB had trace element profiles that resulted in particularly poor classification success. Hatchlings from DEC, BI, and GOB fared better with 50%, 60%, and 83% correct classification, respectively. Importantly, although hatchlings from one of the two closed regions (MP) had very poor classification success, hatchlings from the other closed region (GOB) were allocated with very high classification accuracy, effectively allowing the discrimination between those two key regions of interest. Additionally, very few individuals were misclassified between GOB and MP (Table 2). Ranking of the five trace elements using the Gini importance measure suggests that the most important variable for hatching site classification is Sr, followed by Ba, Mn, Mg, and finally Li (Fig. 4). The Gini variable importance curve does not drop off sharply, indicating significant discrimination ability is likely contributed by all elements.

Using the hatchling allocation rules generated with the random forests, the 152 adults caught throughout the eastern and southeastern waters of Tasmania were classified as originating from one of the six sampled natal regions. We examined and present classifications for every observation and also only those classifications where the probability of class membership exceeded 0.5 (Table 3). Based on elemental analysis, the majority of adults caught from across eastern and southeastern Tasmania were sourced from GOB, with each region indicating at least 55% contribution from GOB (Table 3). Other than the 79.7%–84.4% of the individuals caught in GOB being sourced from GOB, no other regions showed evidence of self-recruitment at a greater than 0.5 level of probability (Table 3). Both GOB and MP are considered the major spawning grounds for southern calamary, although MP appears to be largely supplied by adults from GOB. There was evidence of individuals moving substantial distances; for example, between 55% and 72% of adults caught in TP were classified as originating in GOB, and both GOB and MP showed indications of small contributions from BI. Over two-thirds of the adults were allocated to a natal region with greater than 0.5 probability (Fig. 5), suggesting that we have likely characterized trace element signatures from the major spawning regions across eastern and southeastern Tasmania.

Discussion

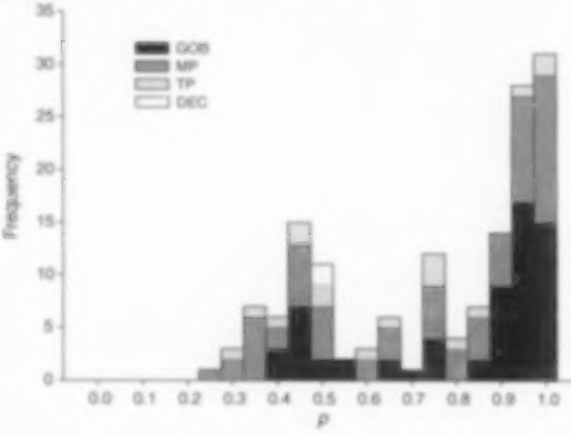
This study used trace element profiles of statoliths to explore the extent of self-seeding, the degree of connectivity among local populations, and the extent of dispersal of hatchlings in southern calamary (*S. australis*). We demonstrated that hatchlings from some regions had distinctive differences in statolith trace element profiles, which allowed us to discriminate among key spawning areas, including two regions closed to commercial fishing during the main spawning period of southern calamary (GOB and MP). By comparing hatchling trace element profiles with those of spawning adults captured at the same locations in the following year, we identified the major source of southern calamary recruits over a large proportion of the east and southeast Tasmanian coast and estimated the relative contribution of different natal areas to the fished adult population. Extensive examinations of southern calamary age and growth from within these re-

Table 3. The percentage (and number in parentheses) of adult *Sepioteuthis australis* that were allocated to one of six hatching regions, with allocations greater than 0.5 probability specified separately, from the random forest analysis.

		Classified to natal region					Classified to natal region with $P > 0.5$		
Capture location	*N*	GOB	MP	DEC	FHB	BI	GOB	FHB	BI
GOB	64	84.4% (54)	—	—	3.1% (2)	12.5% (8)	79.7% (51)	—	1.6% (1)
MP	68	69.1% (47)	5.9% (4)	—	—	25.0% (17)	61.8% (42)	—	5.9% (4)
TP	18	72.2% (13)	5.5% (1)	5.5% (1)	5.5% (1)	11% (2)	55.5% (10)	5.5% (1)	—
DEC	2	100.0% (2)	—	—	—	—	—	—	—
Total	152								

Note: Shaded cells indicate self-recruitment.

Fig. 5. Probability of adults caught from GOB, MP, TP, and DEC being allocated back to a natal region with the random forests analysis using Li, Mn, Mg, Ba, and Sr as classifiers.



gions provide strong evidence that these adults correspond to those that would have hatched at the time our hatchling samples were collected (Pecl et al. 2004). To our knowledge, this is the first study to use trace element profiles of hatchling and adult squid to successfully directly estimate the extent of population connectivity among different spawning populations. As with the majority of studies utilizing trace element analysis to examine population connectivity, we have not been able to sample all possible natal areas. However, we have 15 years of extensive fishery-independent and fishery-dependent surveys that support the selection of our sample locations as the main spawning areas for southern calamary on the east and southeast coast of Tasmania (e.g., Moltschaniwskyj and Pecl 2003, 2007).

This study revealed an important source of recruits to coastal populations of southern calamary. GOB likely contributed at least 55% and up to 84% of the fished adults caught from along the east and southeast Tasmanian coast, and this was the only region with any evidence of self-recruitment. Extensive acoustic tracking and t-bar tagging of adult calamary in GOB and MP, undertaken in the same year as the adults were obtained for the current study, allowed us to determine the phase of the life cycle that movement among regions occurs (Pecl et al. 2006). Reproductively mature adult squid spend the last 2–4 months of their life moving extensively within spawning regions (e.g., within GOB), but not moving between them (e.g., from GOB to MP or vice versa; Pecl et al. 2006). Thus, the movement of individuals originating from GOB occurs during the subadult period and not as adult migration. Southern populations appeared to be substantially augmented by very young individuals from GOB and are therefore likely dependent on the continued persistence of recruitment from this region. This has major implications for the assessment of the potential benefits and risks of given management strategies, including the designation of the location and timing of closed areas, analogous to issues associated with optimal marine reserve design.

Elemental analysis of southern calamary statoliths had sufficient resolution to allow discrimination of the natal source of hatchlings on a spatial scale relevant to management; however, there was poor spatial discrimination among some regions. Using trace elements in open marine systems or with nondiadromous species is difficult because of subtler gradients in the properties of the water compared with stronger elemental signals generated within river and estuarine systems (Thorrold et al. 2007). Despite this, over two-thirds of the adult calamary were able to be allocated to a natal region with reasonable confidence, suggesting we have been able to describe the major population linkages occurring over a broad section of the Tasmanian coast. The individuals that were unable to be allocated to one of the sampled natal areas may be from poorly discriminated hatching regions, areas or times that were not sampled, or individuals with unclear elemental profiles. Greater resolution of population linkages may be obtained by combining trace element analyses with other approaches, such as bacterial, parasites, morphology, otolith shape, and lectins used as specific gene markers to characterize frequencies of blood groups (Arrizabalaga et al. 2004), among others. Genetic analyses, like allozyme electrophoresis (Triantafillos and Adams 2001) and comparison of microsatellite allele frequencies (Shaw et al. 1999; 2004) can also produce dispersal estimates; however, these reflect average movement over evolutionary time scales (Peery et al. 2006).

Any exchange of individuals among spawning aggregations will potentially alter the genotypic composition and biological characteristics of the next generation. Furthermore, in very short-lived species with no mixing of generations, migration could very quickly change the life history characteristics of a population. Quantifying migration rates is therefore only the first step in truly understanding the role of population connectivity in modifying the structure and dynamics of populations. Several questions remain to be answered: What biological characteristics allow individuals to successfully migrate between local groups, and are these characteristics spatially and temporally stable? Do migrants contribute to the reproductive output of the population? What influence does this have on the next generation? Effective dispersal, where individuals survive to reproduce in the new environment, is the primary determinant influencing demography and colonization on ecological time scales and local adaptation, speciation, and extinction on evolutionary time scales (Lester et al. 2007). The outcomes from this study effectively provides the groundwork in establishing evidence of self-recruitment in a short-lived marine species and as such provides a model that allows a quantitative assessment of migration and movement as processes determining population structure and dynamics.

Acknowledgements

We thank Sarah Gilbert for assistance with the trace element procedures, Arko Lucieer and Hugh Pederson for earlier discussion on the statistical procedures, and Bronwyn Gillanders for general advice on the use of trace element analysis. We also extend thanks to the southern calamary fishers who provided adult samples and staff from the Tasmanian Aquaculture and Fisheries Institute who assisted with dive surveys for the collection of southern calamary hatchlings. This project was funded by the Australian Research Council LP0347556 awarded to GTP, the Fishwise Community Grant Scheme, and the Department of Primary Industry, Parks and the Environment with the support of Tasmanian Seafood Industry Council.

References

Arrizabalaga, H., Costas, E., Juste, J., González-Garcés, A., Nieto, B., and López-Rodas, V. 2004. Population structure of albacore *Thunnus alalunga* inferred from blood groups and tag–recapture analyses. Mar. Ecol. Prog. Ser. **282**: 245–252. doi:10.3354/meps282245.

Becker, B.J., Levin, L.A., Fodrie, F.J., and McMillan, P.A. 2007. Complex larval connectivity patterns among marine invertebrate populations. Proc. Natl. Acad. Sci. U.S.A. **104**(9): 3267–3272. doi:10.1073/pnas.0611651104. PMID:17360636.

Begg, G., Friedland, K., and Pearce, J. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. Fish. Res. **43**(1-3): 1–8. doi:10.1016/S0165-7836(99)00062-4.

Boinee, P., De Angelis, A., and Foresti, G.L. 2006. Meta random forests. Int. J. Comput. Int. **2**(3): 138–147.

Breiman, L. 2001. Random forests. Mach. Learn. **45**(1): 5–32. doi:10.1023/A:1010933404324.

Breiman, L., Friedman, J., Stone, C.J., and Olshen, R.A. 1984. Classification and regression trees. Chapman and Hall/CRC, Boca Raton, Fla.

Brophy, D., Jeffries, T.E., and Danilowicz, B.S. 2004. Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological, or structural origins. Mar. Biol. **144**(4): 779–786. doi:10.1007/s00227-003-1240-3.

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. **188**: 263–297. doi:10.3354/meps188263.

Cowen, R.K., and Sponaugle, S. 2009. Larval dispersal and marine population connectivity. Annu. Rev. Mar. Sci. **1**(1): 443–466. doi:10.1146/annurev.marine.010908.163757. PMID:21141044.

Doubleday, Z., Belton, D., Pecl, G., and Semmens, J. 2008. Quantitative elemental imaging of octopus stylets using PIXE and the nuclear microprobe. Nucl. Instrum. Methods Phys. Res. B, **266**(1): 67–72. doi:10.1016/j.nimb.2007.10.027.

Elsdon, T.S., and Gillanders, B.M. 2005. Alternative life-history patterns of estuarine fish: barium in otoliths elucidates freshwater residency. Can. J. Fish. Aquat. Sci. **62**(5): 1143–1152. doi:10.1139/f05-029.

Fogarty, M.J., and Botsford, L.W. 2007. Population connectivity and spatial management of marine fisheries. Oceanography, **20**: 112–123.

Friedman, J.H. 2001. Greedy function approximation: a gradient boosting machine. Ann. Stat. **29**(5): 1189–1232. doi:10.1214/aos/1013203451.

Gillanders, B.M. 2005. Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. Estuar. Coast. Shelf Sci. **64**(1): 47–57. doi:10.1016/j.ecss.2005.02.005.

Gillanders, B.M., and Kingsford, M.J. 2000. Elemental fingerprints of otoliths of fish may distinguish estuarine 'nursery' habitats. Mar. Ecol. Prog. Ser. **201**: 273–286. doi:10.3354/meps201273.

Gillanders, B.M., Sanchez-Jerez, P., Bayle-Sempere, J., and Ramos-Espla, A. 2001. Trace elements in otoliths of the two-banded bream from a coastal region in the south-west Mediterranean: Are there differences among locations? J. Fish Biol. **59**(2): 350–363. doi:10.1111/j.1095-8649.2001.tb00135.x.

Havenhand, H. 1995. Evolutionary ecology of larval types. *In* Ecology of marine invertebrate larvae. *Edited by* L. McEdward. CRC Press, Boca Raton, Fla. pp. 79–122.

Hilborn, R., Quinn, T.P., Schindler, D.E., and Rogers, D.E. 2003. Biocomplexity and fisheries sustainability. Proc. Natl. Acad. Sci. U.S.A. **100**(11): 6564–6568. doi:10.1073/pnas.1037274100. PMID:12743372.

Holmes, S., Witbaard, R., and van der Meer, J. 2003. Phenotypic and genotypic population differentiation in the bivalve mollusc *Arctica isalandica*: results from RAPD analysis. Mar. Ecol. Prog. Ser. **254**: 163–176. doi:10.3354/meps254163.

Hunsicker, M.E., Essington, T.E., Watson, R., and Sumaila, U.R. 2010. The contribution of cephalopods to global marine fisheries: Can we have our squid and eat them too? Fish Fish. **11**(4): 421–438.

Ikeda, Y., Yatsu, A., Arai, N., and Sakamoto, W. 2002. Concentration of statolith trace elements in the jumbo flying squid during El Niño and non-El Niño years in the eastern Pacific. J. Mar. Biol. Assoc. U.K. **82**(5): 863–866. doi:10.1017/S0025315402006264.

Izenman, A.J. 2008. Modern multivariate statistical techniques: regression, classification, and manifold learning. Springer, New York.

Lester, S.E., Ruttenberg, B.I., Gaines, S.D., and Kinlan, B.P. 2007. The relationship between dispersal ability and geographic range size. Ecol. Lett. **10**(8): 745–758. doi:10.1111/j.1461-0248.2007.01070.x. PMID:17594430.

Liaw, A., and Wiener, M. 2002. Classification and regression by randomForest. R News, **2**(3): 18–22.

Lipinski, M.R., and Durholtz, M.D. 1994. Problems associated with ageing squid from their statoliths: towards a more structured approach. Antarct. Sci. **6**(2): 215–222. doi:10.1017/S0954102094000337.

Longerich, H.P., Jackson, S.E., and Günther, D. 1996. Laser ablation inductively coupled plasma mass spectrometric transient signal data acquisition and analyte concentration calculation. J. Anal. At. Spectrom. **11**(9): 899–904. doi:10.1039/ja9961100899.

Moltschaniwskyj, N.A., and Pecl, G.T. 2003. Small-scale spatial and temporal patterns of egg production by the temperate loliginid squid *Sepioteuthis australis*. Mar. Biol. **142**: 509–516.

Moltschaniwskyj, N.A., and Pecl, G.T. 2007. Spawning aggregations of squid (*Sepioteuthis australis*) populations: a continuum of 'micro-cohorts'. Rev. Fish Biol. Fish. **17**: 183–195. doi:10.1007/s11160-006-9025-7.

Moltschaniwskyj, N.A., Pecl, G.T., Lyle, J., Haddon, M., and Steer, M.A. 2003. Population dynamics and reproductive ecology of the southern calamary (*Sepioteuthis australis*) in Tasmania. FRDC Final Rep. 2000/121. Tasmanian Aquaculture and Fisheries Institute, Hobart, Tasmania, Australia.

Pecl, G. 2001. Flexible reproductive strategies in tropical and temperate *Sepioteuthis* squids. Mar. Biol. **138**(1): 93–101. doi:10.1007/s002270000452.

Pecl, G.T., and Jackson, G.D. 2008. The potential impacts of climate change on inshore squid: biology, ecology and fisheries. Rev. Fish Biol. Fish. **18**(4): 373–385. doi:10.1007/s11160-007-9077-3.

Pecl, G.T., Moltschaniwskyj, N.A., Tracey, S., and Jordan, A. 2004. Inter-annual plasticity of squid life-history and population structure: ecological and management implications. Oecologia, **139**(4): 515–524. doi:10.1007/s00442-004-1537-z. PMID:15054657.

Pecl, G.T., Tracey, S.R., Semmens, J.M., and Jackson, G.D. 2006. Use of acoustic telemetry for spatial management of southern calamary *Sepioteuthis australis*, a highly mobile inshore squid species. Mar. Ecol. Prog. Ser. **328**: 1–15. doi:10.3354/meps328001.

Peery, M.Z., Becker, B.H., and Beissinger, S.R. 2006. Combining demographic and count-based approaches to identify source–sink dynamics of a threatened seabird. Ecol. Appl. **16**(4): 1516–1528. doi:10.1890/1051-0761(2006)016[1516:CDACAT]2.0.CO;2. PMID:16937815.

R Development Core Team. 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org/.

Ruttenberg, B.I., Hamilton, S.L., Hickford, M.J.H., Paradis, G.L., Sheehy, M.S., Standish, J.D., Ben-Tzvi, O., and Warner, R.R. 2005. Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. Mar. Ecol. Prog. Ser. **297**: 273–281. doi:10.3354/meps297273.

Shaw, P., Pierce, G., and Boyle, P. 1999. Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. Mol. Ecol. **8**(3): 407–417. doi:10.1046/j.1365-294X.1999.00588.x.

Shaw, P.W., Arkhipkin, A.I., Adcock, G.J., Burnett, W.J., Carvalho, G.R., Scherbich, J.N., and Villegas, P.A. 2004. DNA markers indicate that distinct spawning cohorts and aggregations of Patagonian squid, *Loligo gahi*, do not represent genetically discrete subpopulations. Mar. Biol. **144**(5): 961–970. doi:10.1007/s00227-003-1260-z.

Steer, M.A., Moltschaniwskyj, N.A., and Jordan, A.R. 2003*a*. Embryonic development of southern calamary (*Sepioteuthis australis*) within the constraints of an aggregated egg mass. Mar. Freshw. Res. **54**(3): 217–226. doi:10.1071/MF02107.

Steer, M.A., Pecl, G.T., and Moltschaniwskyj, N.A. 2003*b*. Are bigger calamary *Sepioteuthis australis* hatchlings more likely to survive? A study based on statolith dimensions. Mar. Ecol. Prog. Ser. **261**: 175–182. doi:10.3354/meps261175.

Thorrold, S.R., Zacherl, D.C., and Levin, L.A. 2007. Population connectivity and larval dispersal using geochemical signatures in calcified structures. Oceanography, **20**(3): 80–89.

Triantafillos, L., and Adams, M. 2001. Allozyme analysis reveals a complex population structure in the southern calamary *Sepioteuthis australis* from Australia and New Zealand. Mar. Ecol. Prog. Ser. **212**: 193–209. doi:10.3354/meps212193.

Warner, R.R., Hamilton, S.L., Sheehy, M.S., Zeidberg, L.D., Brady, B.C., and Caselle, J.E. 2009. Geographic variation in natal and early larval trace-elemental signatures in the statoliths of the market squid *Doryteuthis* (formerly *Loligo*) *opalescens*. Mar. Ecol. Prog. Ser. **379**: 109–121. doi:10.3354/meps07903.

Zacherl, D.C., Manriquez, P.H., Paradis, G., Day, R.W., Castilla, J.C., Warner, R.R., Lea, D.W., and Gaines, S.D. 2003. Trace elemental fingerprinting of gastropod statoliths to study larval dispersal trajectories. Mar. Ecol. Prog. Ser. **248**: 297–303. doi:10.3354/meps248297.

Does increased information about reproductive potential result in better prediction of recruitment?

M. Joanne Morgan, Alfonso Perez-Rodriguez, and Fran Saborido-Rey

Abstract: The relationship between stock size and recruitment is an essential element in the understanding of the productivity of a population. However, predicting the number of recruits produced by a population has proven to be a difficult challenge. This may in part be a result of poor estimation of reproductive potential (RP). We determined if including increased information on reproductive biology in indices of RP results in better predictions of recruitment. We investigated some of the conditions that lead to better (or worse) recruitment prediction when more biologically complex indices of RP are used. Data from four populations in the Northwest Atlantic were examined: southern Grand Bank (NAFO Division 3NO) cod (*Gadus morhua*), Flemish Cap (NAFO Division 3M) cod, Newfoundland (NAFO Division 3LNO) American plaice (*Hippoglossoides platessoides*), and Greenland halibut (*Reinhardtius hippoglossoides*) (NAFO Subarea 2 + Division 3KLMNO). Stock–recruit models paired with complex indices of RP gave a better estimate of recruitment in slightly more than half of the tests conducted. When there were larger trends in the reproductive biology (maturity at age, sex ratio and egg production), more complex indices of RP were more likely to provide a better estimate of recruitment.

Résumé : La relation entre la taille d'un stock et le recrutement est un élément essentiel pour la compréhension de la productivité d'une population. Cependant, la prédiction du nombre de recrues produites par la population s'est avérée être un défi difficile. Ce peut en partie être le résultat d'une mauvaise estimation du potentiel reproductif (RP). Nous avons déterminé si l'addition d'information supplémentaire sur la biologie de la reproduction dans les indices de RP produit de meilleures prédictions du recrutement. Nous avons étudié certaines des conditions qui entraînent une meilleure (ou moins bonne) prédiction du recrutement lorsque des indices plus biologiquement complexes de RP sont utilisés. Nous avons examiné les données provenant de quatre populations du nord-ouest de l'Atlantique, les morues (*Gadus morhua*) du Grand Banc sud (NAFO Division 3NO), les morues de Flemish Cap (NAFO Division 3M), les plies canadiennes (*Hippoglossoides platessoides*) de Terre-Neuve (NAFO Division 3LNO) et les flétans du Groenland (*Reinhardtius hippoglossoides*) (NAFO sous-région 2 + Division 3KLMNO). Les modèles stocks-recrues appariés à des indices complexes de PR donnent une meilleure estimation du recrutement dans un peu plus de la moitié des tests. Lorsqu'il y a des tendances plus marquées dans la biologie de la reproduction (maturité en fonction de l'âge, proportion des sexes, production des œufs), les indices plus complexes de PR sont plus susceptibles de fournir une meilleure estimation du recrutement.

[Traduit par la Rédaction]

Introduction

Predicting the number of recruits produced by a population has proven to be a difficult challenge in fisheries science. Yet the relationship between stock size and recruitment is an essential element in the understanding of the productivity of a population (Hilborn and Walters 1992; Needle 2002). The stock–recruit (S-R) relationship will determine the level of fishing mortality a population can sustain. As a result, S-R relationships form the basis for the setting of many reference points in fisheries management (Sissenwine and Shepherd 1987; Mace 1994).

The reproductive potential (RP) of a population is its potential to produce recruitment and is determined by a combination of factors including maturation, sex ratio, and fecundity (Trippel 1999). The actual recruitment that arises from this RP is greatly influenced by environmental impacts on egg and larval survival (Shepherd et al. 1984; Cushing 1996). However, the underlying RP also plays a role (Myers and Barrowman 1996). In fact, the impact of poor environmental conditions on recruitment may be exacerbated at low levels of RP (Brander 2005). The importance of RP means that difficulty in estimating recruitment may in part be a result of poor estimation of RP. Error in the measurement of RP or failure to use an index of RP that adequately reflects a population's actual RP can obscure any S-R relationship (Walters and Ludwig 1981; Cadigan 2009). RP is often expressed as a metric that does not account for variation in maturation and sex ratio. Changes in fecundity are rarely incorporated into indices of RP (Morgan 2008; Marshall 2009). However, it is clear that populations can exhibit large

Received 25 June 2010. Accepted 24 March 2011. Published at www.nrcresearchpress.com/cjfas on 19 August 2011.
J21893

Paper handled by Associate Editor C. Tara Marshall.

M.J. Morgan. Fisheries and Oceans Canada, P.O. Box 5667, St. John's, NL A1C 5X1, Canada.
A. Perez-Rodriguez and F. Saborido-Rey. Institute of Marine Research, CSIC, Vigo, Spain.

Corresponding author: M. Joanne Morgan (e-mail: Joanne.Morgan@dfo-mpo.gc.ca).

Can. J. Fish. Aquat. Sci. **68**: 1361–1368 (2011) doi:10.1139/F2011-049 Published by NRC Research Press

variation, and more importantly trends over time, in these biological characteristics (Jørgensen 1990; Jakobsen and Ajiad 1999; Rideout and Morgan 2007). It is also clear that using more complex indices of RP that account for this variation in reproductive biology can result in an altered perception of population productivity and hence sustainable levels of fishing (Scott et al. 2006; Marshall et al. 2003; Morgan et al. 2009).

Since biological factors incorporated into more complex indices of RP are themselves estimated with error, their use may simply add more error to the estimate of RP. Therefore, it is uncertain whether they will result in an improved S-R relationship or ability to predict recruitment. In addition, environmental influences on offspring survival may make the detection of RP affects difficult (Brunel 2010). Only a few studies have examined whether more complex indices of RP improve the ability to predict recruitment, and the results have been equivocal (Marteinsdottir and Thorarinsson 1998; Kraus et al. 2002; Marshall et al. 2006). Given the importance of the S-R relationship to our understanding of population productivity, an increase in error in recruitment prediction as a result of using poorly estimated biological characteristics in our construction of RP would be undesirable (Morgan 2008; Marshall 2009; Brunel 2010).

The purpose of this study was to determine if more complex indicators of RP result in better predictions of recruitment. We also investigate some of the conditions that lead to better (or worse) recruitment prediction when more biologically complex indicators of RP are used. We examine four indices of RP in four populations of groundfish in the Northwest Atlantic.

Materials and methods

Data from four populations in the Northwest Atlantic off the east coast of Canada were examined: southern Grand Bank cod (*Gadus morhua*) in Northwest Atlantic Fisheries Organization (NAFO) Division 3NO, Flemish Cap cod in NAFO Division 3M, Newfoundland American plaice (*Hippoglossoides platessoides*) in NAFO Division 3LNO, and Greenland halibut (*Reinhardtius hippoglossoides*) in NAFO Subarea 2 + Division 3KLMNO. Population numbers at age and masses at age were taken from recent stock assessments of these populations: southern Grand Bank cod in Morgan et al. (2007); Greenland halibut in Healey and Mahé (2008); American plaice in Dwyer et al. (2009); Flemish Cap cod in González-Troncoso and Fernández (2009). All population models used a plus group except for southern Grand Bank cod. The age range in the assessment for southern Grand Bank cod was 2–12, for Flemish Cap cod 1–8+, for 3LNO American plaice 5–15+, and for Greenland halibut 1–14+.

Data on maturity, sex ratio, and fecundity were collected from research vessel bottom trawl surveys catches. The American plaice and southern Grand Bank cod data were collected during Canadian spring surveys mainly from 1975 to 2008, although some data from as early as 1965 were included. Maturity stage was determined macroscopically, which is generally easy at this time of the year. Fecundity data were available from only a few years (see below). For both of these populations, the surveys covered their whole distribution. For Greenland halibut, Canadian research vessel bottom trawl surveys were conducted in the fall from 1978 to 2007. Survey data from Divisions 2J and 3K only were used, as these areas had the most consistent coverage of the deep water areas inhabited by Greenland halibut. Maturities were determined macroscopically, which is generally considered accurate, given that gonads have begun to develop at this time of year for the next spawning season. The bottom trawl survey does not have good catchability of large fish. In addition, starting in the early 1990s the frequency of older and larger fish declined substantially, meaning that there are few data from adults, probably increasing the variability in the estimates of maturity at age, and possibly sex ratio. A single fecundity relationship for Greenland halibut was taken from the literature (Bowering 1980). Flemish cap cod survey data were available from Canada (1978–1985, February) and Spain (1988–2008, July). In both cases the entire distribution of the population was surveyed. The Canadian survey relied on macroscopic determination of maturity stage, while the Spanish survey used histology to determine maturity stage. The latter is generally more accurate, particularly in the summer, but sample size is low. In autumn 1996, a survey of the Flemish Cap was conducted by Canada that used the macroscopic method for staging maturity. This allowed a comparison with the Spanish survey conducted in July of 1997. Estimates of length at 50% maturity were similar from both surveys (Spanish = 45.8 cm, Canadian = 46.2 cm), indicating that changes in maturity over time are real and not a result of the change in methodology. For Flemish Cap cod, a single fecundity length relationship was taken from Rideout and Morgan (2009).

Proportion mature at age was estimated by cohort using generalized linear models with a logit link function and binomial error. Age was treated as a continuous variable, since in general it is not possible for there to be a lower proportion of adults at age $a + 1$ than at age a (Morgan and Colbourne 1999). For cohorts where there was no significant model fit to the data, the average of estimates from adjacent cohorts or of the three closest cohorts were used. All ages were used in the fitting. Sample sizes per cohort were generally large, ranging from 87 to 835 for southern Grand Bank cod, from 105 to 3644 for Flemish Cap cod, from 272 to 1908 for American plaice, and from 219 to 1356 for Greenland halibut.

Sex ratio (proportion female) at age was also estimated using generalized linear models with a logit link function and binomial error. These models had the form sex ratio = age + cohort, where age and cohort were both class variables. In this case age was treated as a class variable, since there is no a priori reason to believe that sex ratio would change continuously across age (Morgan and Brattey 2005). Only ages for a cohort with at least five observations (10 for southern Grand Bank cod) were used in the fitting. Sample sizes for a cohort ranged from 119 to 1270 for southern Grand Bank cod, from 40 to 7298 for Flemish Cap cod, 1041 to 2852 for American plaice, and from 753 to 1527 for Greenland halibut. Similar to proportion mature at age, for cohorts where there was no significant fit to the data, averaging was used to produce the sex ratio. For Greenland halibut, a single model did not seem to adequately describe the changes in sex ratio (based on examination of patterns in the residuals), and so two separate models were fit: one using the 1969–1980 cohorts and the second using the 1981–1999 cohorts.

Far fewer data were available for the estimation of fecundity. For Flemish Cap cod and Greenland halibut, only single fecundity length relationships were available, which were applied to mean length at age to produce egg production at age. Yearly relationships of fecundity and mass were available for southern Grand Bank cod in 1990, 1993, 1994, 1996, 1998–2003 and for American plaice in 1993–1998 and 2005. These were applied to mean mass at age to produce fecundity at age. For southern Grand Bank cod and American plaice in years for which no relationship was available, a single fecundity mass relationship using all fecundity data was used. The length at age (for Flemish Cap cod and Greenland halibut) or mass at age (for Grand Bank cod and American plaice) used were the beginning of the year estimates from the assessment and should be similar to spawning time for spring spawning fish. However, length and mass at age were for sexes combined and so could deviate from true female mass or length. Fecundity is determined mostly by size (length and mass), and so age was not included in the modeling (Lambert et al. 2003).

To examine some of the potential reasons for any differences between the populations in the ability of different indices of RP to predict recruitment, we examined trends in maturity at age, sex ratio, and fecundity. We used a metric for each that reflected changes over time. For maturity at age this was age at 50% maturity. For sex ratio it was the parameter estimate for the cohort effect. For fecundity we summed, for each year, the number of eggs produced by one fish at each age. We then standardized this by dividing the egg production for each year by the average across all years for the population.

Four indices of RP were calculated using the above data. The first estimate of RP (+BIO) did not incorporate fecundity and assumed no change in the maturity schedule of the fish, or in the sex ratio, and that all fish older than a particular age are mature.

$$(1)\qquad +\mathrm{BIO} = \sum_{a=i}^{j} N_{ay} M_{ay}$$

where N_{ay} is the population number at age a in year y, M_{ay} the mass at age a in year y, j is the maximum age in the sequential population analyses for the population, and i is 6, 5, 9, and 10 (the age at which all fish are assumed to be mature) for southern Grand Bank cod, Flemish Cap cod, American plaice, and Greenland halibut, respectively. This index is simply the sum of the biomass of all fish greater than a given age.

The second estimate, spawning stock biomass (SSB), incorporates the estimated proportion mature at age for each cohort, that is, variable rather than constant maturity at age.

$$(2)\qquad \mathrm{SSB} = \sum_{a=i}^{j} N_{ay} M_{ay} \mathrm{PM}_{ay}$$

where i is the first age in the virtual population analysis (VPA) for the stock, PM_{ay} is the proportion mature at age a in year y.

In the third estimate of RP, we applied the sex ratios estimated along with the variable estimates of proportion mature at age to obtain a female-only spawning stock biomass (FSB):

$$(3)\qquad \mathrm{FSB} = \sum_{a=i}^{j} N_{ay} M_{ay} \mathrm{PM}_{ay} \mathrm{SR}_{ay}$$

where SR_{ay} is the sex ratio (i.e., proportion female) at age a in year y, and the other symbols are as defined above.

The fourth estimate of RP incorporated estimates of fecundity at age and is an estimate of total egg production (TEP).

$$(4)\qquad \mathrm{TEP} = \sum_{a=i}^{j} N_{ay} \mathrm{PM}_{ay} \mathrm{SR}_{ay} E_{ay}$$

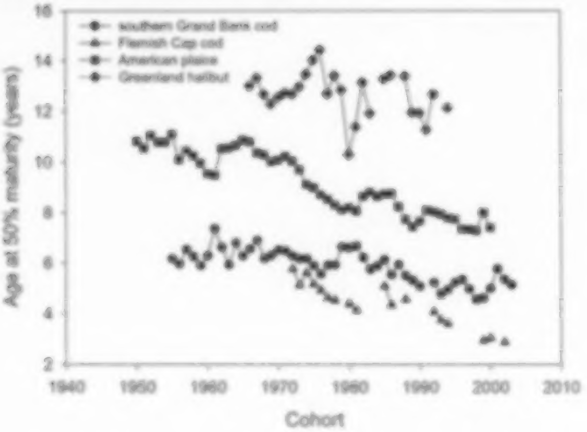
where E_{ay} is the number of eggs produced (i.e., potential fecundity) per female at age a in year y, and the other symbols are as defined above.

To examine the differences in indices of RP, we standardized each index to its mean and variance. We then plotted each index of RP for a population on the same plot to see how they differed from one another.

Three S-R models were fit to each index of RP. These were both parametric (Beverton–Holt and Ricker) and nonparametric (generalized additive models). The first two were based on S-R theory (Beverton and Holt 1957; Ricker 1954) and assume a particular functional relationship. Generalized additive models are also used to model S-R relationships (Cardinale and Arrhenius 2000; Lee et al. 2009; Brunel 2010) but do not impose a functional form. For each S-R model, the RP that gave the best predictor of recruitment was determined to see if predictability was improved for a given S-R model by using more complex indices of RP. Different indices of RP may not just alter the fit of a particular S-R model but may actually change the shape of the S-R relationship from one best described by one model to one best described by a different model. Poor ability to predict recruitment could be due to inappropriate model choice rather than a real change in predictive ability. The issue of potential bias of using a single S-R model is accounted for by examining three different S-R models. The combination of S-R model and RP index giving the best prediction of recruitment was determined.

There were two tests of the ability to predict recruitment that were similar to the methods used by Lee et al. (2009). The first was the ability to predict 10% of the data that were selected at random and removed and the model then fit without these data. This first procedure was repeated 100 times for each S-R model for each index of RP for each population. In six cases out of 48 (four stocks × four indices of RP × three S-R models), there were problems with convergence and multiple runs were conducted to achieve 100 successful model fits. Results were accumulated from each run until 100 were complete. The second test was the ability to predict the most recent 10% of the data when these data were not used in the model fitting. Stock assessments are often tasked with examining sustainable fishing levels in the short to medium term. If recent recruitment levels are likely to be indicative of recruitment in the near future, then the ability to predict recent values should not be compromised. An additional reason for this second test is that leaving out the most recent data is a technique often used in evaluating the ability to predict (Lee et al. 2009).

Fig. 1. Age at 50% maturity estimated by cohort for each of the study populations. southern Grand Bank cod, circles; Flemish Cap cod, triangles; American plaice, squares; Greenland halibut, diamonds.



In both cases the measure of predictive ability was the mean absolute error (MAE), where

$$(5)\qquad \mathrm{MAE} = \frac{\sum_{i=1}^{n}\left|Y_i - \hat{Y}\right|}{n}$$

that is, the average of the absolute difference between the observed and predicted recruitments. For each of these three tests for a population, we found the S-R model with the lowest MAE for each RP and then for each test the RP with the lowest MAE.

Results

All of the populations exhibited substantial variation in age at 50% maturity, with age at maturity being lower for more recent cohorts (Fig. 1). There was a clear declining trend throughout the time series for southern Grand Bank cod, Flemish Cap cod, and American plaice. Linear regressions showed that the decline was significant ($p < 0.0001$) and steepest for Flemish Cap cod and American plaice, with slopes of –0.08 for each population. The decline was less for southern Grand Bank cod at –0.04, but still significant ($p < 0.0001$). For Greenland halibut the slope was –0.03 and the regression was not significant.

The effect of cohort on sex ratio was also highly variable over time for most stocks (Fig. 2). The clearest trends were again for Flemish Cap cod and American plaice, and the least variation was found for Greenland halibut. The greatest range in parameter estimates was for American plaice, but there was no consistent trend over the entire time series for that population. There was a decline to the early 1980s followed by a large increase to 1987 and then a general decline. For Flemish Cap cod, there was an increasing trend (indicating an increasing proportion female) across the whole time series.

Fig. 2. The parameter estimate for the effect of cohort on sex ratio (proportion female) for each of the study populations: (*a*) southern Grand Bank cod, (*b*) Flemish Cap cod, (*c*) American plaice, and (*d*) Greenland halibut.

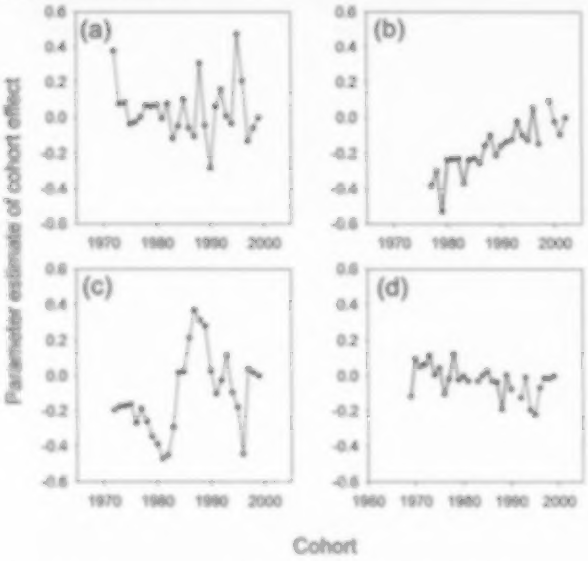
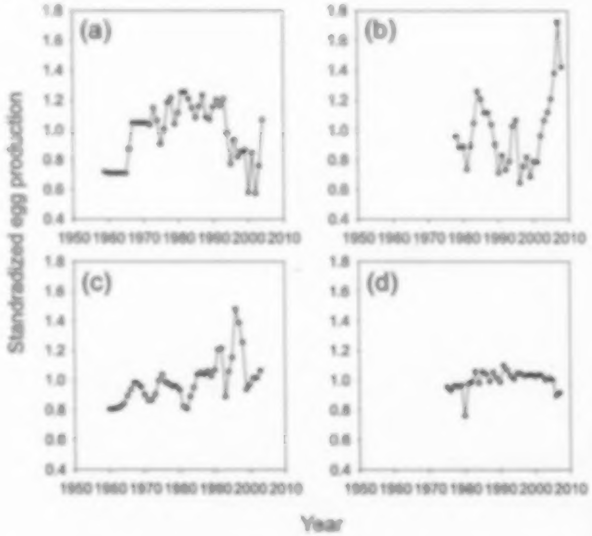
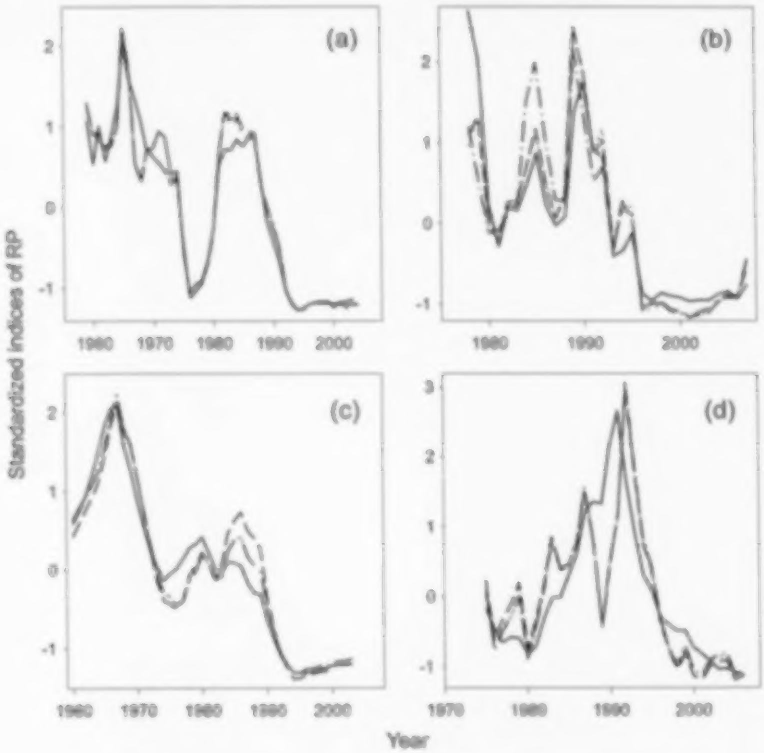


Fig. 3. The standardized egg production for each year for each of the study populations: (*a*) southern Grand Bank cod, (*b*) Flemish Cap cod, (*c*) American plaice, and (*d*) Greenland halibut. For each year, the number of eggs produced by one fish at each age for the age range used in the indices of reproductive potential (RP) was summed. This metric was then standardized by dividing the egg production for each year by the average across all years for the population.



Greenland halibut also showed the least variation in standardized egg production (Fig. 3). The largest variation was found in Flemish Cap cod, which had an increasing trend from 1999 to 2008 over which time egg production more than doubled. American plaice had a general increasing trend in egg production up to 1996, followed by a large decline. Southern Grand Bank cod had higher egg production from

Fig. 4. The standardized indices of reproductive potential (RP) for each year for each of the study populations: (*a*) southern Grand Bank cod, (*b*) Flemish Cap cod, (*c*) American plaice, and (*d*) Greenland halibut. Each index was standardized by its mean and variance. +BIO, solid line; spawning stock biomass (SSB), short dashed line; female spawning stock biomass (FSB), dotted line; total egg production (TEP), dash-dot-dashed line.



the late 1960s to the early 1990s, but there were few trends over the time period.

The more complex indices of RP differed from +BIO for all populations (Fig. 4). They were generally more similar to one another than to +BIO. The relative position of the indices varied over time for all populations, but the pattern was unique to each population. For Greenland halibut, the more complex indices of RP were generally above standardized +BIO in the first part of the time series and lower in the second part of the time series. For Flemish Cap cod, the more complex indices of RP were above +BIO from 1980 to 1995. All indices of RP show a steep increase in the most recent years, but the increase is much greater for the more complex indices of RP. For American plaice, there were three shifts in the position of standardized +BIO relative to all other indices of RP. Since the early 1990s, it has been below TEP and FSB but above SSB. In southern Grand Bank cod, there have also been several shifts in the position of standardized +BIO relative to other indices of RP, but since the early 1990s they have all been quite similar.

When 10% of the data were left out at random 100 times and predicted from the model fit to the remainder of the data, two of the four populations had S-R models combined with more complex indices of RP that gave the lowest MAE (Table 1). These were southern Grand Bank cod for which the lowest S-R–RP index combination was Beverton–Holt with TEP and for Flemish Cap cod Beverton–Holt with FSB. However, the Beverton–Holt fit for southern Grand Bank cod to TEP failed to converge in 17 of the 100 model runs. The best predictor for this stock that did not have convergence problems was the Ricker model fit to SSB. For southern Grand Bank and Flemish Cap cod, more complex indices of RP were also the best predictors for each of the three S-R models, although which index of RP had the lowest MAE was not the same for all models. For both American plaice and Greenland halibut, the +BIO index of RP gave the lowest MAE. This was the case regardless of the S-R model used.

When trying to predict the most recent 10% of the observed data, the +BIO RP was the best predictor for only Greenland halibut (Table 2). For the other three populations, an S-R model paired with a more complex index of RP predicted the most recent observations better: TEP for southern Grand Bank cod, FSB for Flemish Cap cod, and SSB for American plaice. There was some variation in which index of RP had the lowest MAE for each S-R model (except for American plaice, for which SSB had the lowest MAE for all three models). However, for 8 of 12 population S-R model combinations, a more complex index of RP had the lowest MAE.

Discussion

One of the reasons for attempting to produce more biologically realistic indices of RP is the belief that this will lead to a

Table 1. Mean absolute error (MAE) for each index of reproductive potential (RP) for model predictions of 10% of observed recruitments removed at random, 100 times.

	+BIO	SSB	FSB	TEP
Southern Grand Bank cod				
Beverton–Holt	32.68	32.87	32.17	**30.79***
Ricker	33.14	**32.25**	34.60	35.00
GAM	36.24	36.89	38.20	**36.22**
Flemish Cap cod				
Beverton–Holt	18.02	16.08	**15.04***	16.12
Ricker	17.20	19.62	**16.72**	18.37
GAM	18.92	21.96	24.02	**18.57**
American plaice				
Beverton–Holt	**42.12**	48.42	45.37	46.77
Ricker	**37.93**	49.15	42.49	47.73
GAM	**36.43***	50.68	48.80	45.54
Greenland halibut				
Beverton–Holt	**25.29**	28.19	29.58	27.50
Ricker	**20.04**	25.75	24.12	23.32
GAM	**16.87***	29.10	26.14	26.03

Note: For +BIO RP, all fish 6+ for southern Grand Bank cod, 5+ for Flemish Cap cod, 9+ for American plaice, and 10+ for Greenland halibut are considered to be mature. The other indices of RP are spawning stock biomass (SSB), female spawning stock biomass (FSB), and total egg production (TEP). Bold font indicates the RP giving the lowest MAE for each stock–recruitment (S-R) model. For each population, an asterisk (*) indicates the RP index – S-R model combination giving the lowest MAE. GAM, generalized additive model.

Table 2. Mean absolute error (MAE) for model predictions of the most recent 10% of observed recruitments for each index of reproductive potential (RP).

	+BIO	SSB	FSB	TEP
Southern Grand Bank cod				
Beverton Holt	1.33	3.22	14.83	**1.25***
Ricker	**1.33**	2.49	2.38	1.83
GAM	2.31	6.52	6.45	**1.98**
Flemish Cap cod				
Beverton Holt	9.72	8.99	**8.82**	8.89
Ricker	9.77	8.97	8.82	**8.76***
GAM	9.92	9.08	8.85	**8.82**
American plaice				
Beverton Holt	31.22	**28.71***	29.48	29.77
Ricker	33.08	**29.08**	30.17	30.37
GAM	33.83	**30.98**	31.64	31.78
Greenland halibut				
Beverton Holt	33.53	**21.62**	21.85	39.18
Ricker	24.19	**24.07**	24.63	25.50
GAM	**16.96***	41.43	42.43	43.04

Note: For +BIO RP, all fish 6+ for southern Grand Bank cod, 5+ for Flemish Cap cod, 9+ for American plaice, and 10+ for Greenland halibut are considered to be mature. The other indices of RP are spawning stock biomass (SSB), female spawning stock biomass (FSB), and total egg production (TEP). Bold font indicates the RP giving the lowest MAE for each stock–recruitment (S-R) model. For each population, an asterisk (*) indicates the RP index – S-R model combination giving the lowest MAE is shaded. GAM, generalized additive model.

better understanding of population productivity and better prediction of recruitment (Morgan 2008; Marshall 2009). To date, studies have found some examples where S-R relationships were improved and some where they were not (Marteinsdottir and Thorarinsson 1998; Murawski et al. 2001; Marshall et al. 2006). The results of this study were similar with slightly more than half of the tests showing that S-R models paired with more complex indices of RP did better in the prediction of recruitment than using indices of RP that did not account for variation in reproductive biology.

Populations with the greatest trends in maturity, sex ratio, and egg production were the ones where the best predictor of recruitment tended to come from more complex indices of RP. These trends in reproductive biology led to deviation of the more complex indices of RP from +BIO and to a lesser extent from each other. For the population with the greatest trends, Flemish Cap cod, more complex indices of RP were always the better predictors of recruitment. This feature of trends in variation in reproductive biology is likely to be an important factor leading to the need for more complex indices of RP. These trends make indices of RP that do not take variation in reproductive biology into account less reflective of the true RP of the population. In essence it increases the error with which these simple indices of RP measure true RP. This will lead to an obscuring of the relationship between stock and recruit (Walters and Ludwig 1981; Cadigan 2009).

For Greenland halibut, using the simplest index of RP (10+ biomass) resulted in the best predictor of recruitment in all tests. This population showed the least trend in maturity, sex ratio, and fecundity, leading to little trend in the deviation of the constant RP from true RP. The biological factors will always be measured with error, and if there is little variation and (or) trend, incorporating these factors simply adds to the error in the measure of RP rather than capturing any real shift in the true RP of the population. Reducing errors in the measurement and estimation of biological factors may improve the estimation of RP. In the case of Greenland halibut, there is an additional reason why incorporating more biology into the indices of RP did not result in an improved prediction of recruitment. The assessment of this population only estimates numbers at age to age 13 with a plus group at age 14 (Healey and Mahé 2008). Age at 50% maturity in this population is currently age 12. This means that most of the mature biomass is in the plus group. Indices of RP incorporating the measures of reproductive biology will only be minimally affected by any changes in reproductive investment, as most of the change will be "hidden" in the plus group.

Data on the biological factors determining RP were incomplete for all of these populations. None had a complete time series of fecundity so that fecundity in most (or all) years was calculated by using the fecundity–length or fecundity–mass relationship from another year. Changes in fecundity, therefore, were mostly a reflection of changes in growth. In effect, a proxy for fecundity was constructed for those years. If the proxy does not have a strong relationship with fecundity, or if that relationship changes over time, it will not likely lead to an improvement in the index of RP (De Oliveira et al. 2006).

There is also error in estimates of sex ratio and maturity at age in all populations. For example, for Greenland halibut, estimates of maturity and sex ratio at age were complicated by the low catchability of older fish in the survey. For Flemish Cap cod, sample size was low for the estimation of maturity at age for recent cohorts. Despite these issues, in just over half of the tests more complex indices of RP gave a better estimate of recruitment. For populations where complex indices of RP gave better predictions of recruitment, the improvement was often small (small differences in MAE), but there was no sign of deterioration in the ability to predict recruitment. The added biological realism did not come at a price of predictive ability. If better time series of biological factors could be constructed, our ability to predict recruitment may improve further.

The factors required to build more complex indices of RP can themselves provide insight into changes in population productivity. Increased mortality can lead to changes in maturity, fecundity, and sex ratio (Jakobsen and Ajiad 1999; Yoneda and Wright 2004; Olsen et al. 2004). Changes in population abundance can result in density-dependent responses in maturation and fecundity (Rijnsdorp 1993; 1994). Even for those populations where the incorporation of these reproductive characteristics does not now improve our prediction of recruitment, it is important to continue to monitor these factors and to try to improve our understanding of the underlying causes of variation in them.

The index of RP that provides the best prediction of recruitment will be stock specific as seen in this study. It will depend on the amount of temporal variation in reproductive characteristics and on how well these are estimated. It might also change over time as better information over a longer time period is collected. Trend in this variation is more likely to make more complex indices of RP better predictors of recruitment. We should continue to test whether adding biological complexity results in better predictions of recruitment for more populations and for those populations already examined.

Acknowledgements

We thank the many people involved in the collection of these data. We thank J. Brattey, K. Dwyer, P. Pepin, and two anonymous reviewers for valuable comments on an earlier version of this manuscript. This paper is a product of the NAFO Working Group on Reproductive Potential and the EU COST Action FA0601 Fish Reproduction and Fisheries (FRESH).

References

Beverton, R.J.H., and Holt, S.J. 1957. On the dynamics of exploited fish populations. Ministry of Agriculture, Fisheries and Food, London, UK.

Bowering, W.R. 1980. Fecundity of Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), from southern Labrador and southeastern Gulf of St. Lawrence. J. Northwest Atl. Fish. Sci. **1**: 39–43. doi:10.2960/J.v1.a3.

Brander, K.M. 2005. Cod recruitment is strongly affected by climate when stock biomass is low. ICES J. Mar. Sci. **62**(3): 339–343. doi:10.1016/j.icesjms.2004.07.029.

Brunel, T. 2010. Age-structure-dependent recruitment: a meta-analysis applied to northeast Atlantic fish stocks. ICES J. Mar. Sci. **67**(9): 1921–1930. doi:10.1093/icesjms/fsq032.

Cadigan, N.G. 2009. Sensitivity of common estimates of management parameters derived from stock–recruit relationships. Fish. Res. **96**(2–3): 195–205. doi:10.1016/j.fishres.2008.11.003.

Cardinale, M., and Arrhenius, F. 2000. The relationship between stock and recruitment: are the assumptions valid? Mar. Ecol. Prog. Ser. **196**: 305–309. doi:10.3354/meps196305.

Cushing, D.H. 1996. Towards a science of recruitment in fish populations. Ecology Institute, Oldendorf, Germany.

De Oliveira, J.A.A., Roel, B.A., and Dickey-Collas, M. 2006. Investigating the use of proxies for fecundity to improve management advice for western horse mackerel *Trachurus trachurus*. ICES J. Mar. Sci. **63**(1): 25–35. doi:10.1016/j.icesjms.2005.07.006.

Dwyer, K.S., Morgan, M.J., Maddock Parsons, D., Brodie, W.B., and Healey, B.P. 2009. An assessment of American plaice in NAFO Div. 3LNO. Northwest Atlantic Fisheries Organization (NAFO) SCR Doc. 09/35.

González-Troncoso, D., and Fernández, C. 2009. Assessment of the cod stock in NAFO Division 3M. Northwest Atlantic Fisheries Organization (NAFO) SCR Doc 09/34.

Healey, B.P., and Mahé, J-C. 2008. Assessment of Greenland halibut (*Reinhardtius hippoglossoides*) in NAFO Subarea 2 and Divisions 3KLMNO. Northwest Atlantic Fisheries Organization (NAFO) SCR Doc. 08/48.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York.

Jakobsen, T., and Ajiad, A. 1999. Management implications of sexual differences in maturation and spawning mortality of Northeast Arctic cod. J. Northwest Atl. Fish. Sci. **25**: 125–131. doi:10.2960/J.v25.a11.

Jorgensen, T. 1990. Long-term changes in age at sexual maturity of northeast Arctic cod (*Gadus morhua* L.). J. Cons. Cons. Int. Explor. Mer. **46**: 235–248.

Kraus, G., Tomkiewicz, J., and Koster, F.W. 2002. Egg production of Baltic cod (*Gadus morhua*) in relation to variable sex ratio, maturity, and fecundity. Can. J. Fish. Aquat. Sci. **59**(12): 1908–1920. doi:10.1139/f02-159.

Lambert, Y., Yaragina, N.A., Kraus, G., Marteinsdottir, G., and Wright, P.J. 2003. Using environmental and biological indices as proxies for egg and larval production of marine fish. J. Northwest Atl. Fish. Sci. **33**: 115–159. doi:10.2960/J.v33.a7.

Lee, Y.-W., Megrey, B.A., and Macklin, S.A. 2009. Evaluating the performance of Gulf of Alaska walleye Pollock (*Theragra chalcogramma*) recruitment forecasting models using a Monte Carlo resampling strategy. Can. J. Fish. Aquat. Sci. **66**(3): 367–381. doi:10.1139/F08-203.

Mace, P.M. 1994. Relationship between common biological reference points used as thresholds and targets for fisheries management strategies. Can. J. Fish. Aquat. Sci. **51**(1): 110–122. doi:10.1139/f94-013.

Marshall, C.T. 2009. Implementing information on stock reproductive potential in fisheries management: the motivation, challenges and opportunities. *In* Fish reproductive biology. Implications for assessment and management. *Edited by* T. Jakobsen, M.J. Fogarty, B.A. Megrey, and E. Moksness. Wiley-Blackwell West Sussex, UK.

Marshall, C.T., O'Brien, L., Tomkiewicz, J., Köster, F.W., Kraus, G., Marteinsdottir, G., Morgan, M.J., Saborido-Rey, F., Blanchard, J.L., Secor, D.H., Wright, P.J., Mukhina, N.V., and Björnsson, H. 2003. Developing alternative indices of reproductive potential for use in fisheries management: case studies for stocks spanning an information gradient. J. Northwest Atl. Fish. Sci. **33**: 161–190. doi:10.2960/J.v33.a8.

Marshall, C.T., Needle, C.L., Thorsen, A., Kjesbu, O.S., and Yaragina, N.A. 2006. Systematic bias in estimates of reproductive potential of an Atlantic cod (*Gadus morhua*) stock: implications for stock–recruit theory and management. Can. J. Fish. Aquat. Sci. **63**(5): 980–994. doi:10.1139/F05-270.

Marteinsdottir, G., and Thorarinsson, K. 1998. Improving the stock–recruitment relationships in Icelandic cod (*Gadus morhua*) by including age diversity of spawners. Can. J. Fish. Aquat. Sci. **55**(6): 1372–1377. doi:10.1139/cjfas-55-6-1372.

Morgan, M.J. 2008. Integrating reproductive biology into scientific advice for fisheries management. J. Northwest Atl. Fish. Sci. **41**: 37–51. doi:10.2960/J.v41.m615.

Morgan, M.J., and Brattey, J. 2005. Effect of changes in reproductive potential on perceived productivity of three Northwest Atlantic cod (*Gadus morhua*) stocks. ICES J. Mar. Sci. **62**(1): 65–74. doi:10.1016/j.icesjms.2004.10.003.

Morgan, M.J., and Colbourne, E.B. 1999. Variation in maturity at age and size in three populations of American plaice. ICES J. Mar. Sci. **56**(5): 673–688. doi:10.1006/jmsc.1999.0487.

Morgan, M.J., Murphy, E.F., and Brattey, J. 2007. An assessment of the cod stock in NAFO Divisions 3NO. Northwest Atlantic Fisheries Organization (NAFO) SCR Doc. 07/40.

Morgan, M.J., Murua, H., Kraus, G., Lambert, Y., Marteinsdóttir, G., Marshall, C.T., O'Brien, L., and Tomkiewicz, J. 2009. The evaluation of reference points and stock productivity in the context of alternative indices of stock reproductive potential. Can. J. Fish. Aquat. Sci. **66**(3): 404–414. doi:10.1139/F09-009.

Murawski, S.A., Rago, P.J., and Trippel, E.A. 2001. Impacts of demographic variation in spawning characteristics on reference points for fishery management. ICES J. Mar. Sci. **58**(5): 1002–1014. doi:10.1006/jmsc.2001.1097.

Myers, R.A., and Barrowman, N.J. 1996. Is fish recruitment related to spawner abundance? Fish Bull. **94**: 707–724.

Needle, C.L. 2002. Recruitment models: diagnosis and prognosis. Rev. Fish Biol. Fish. **11**(2): 95–111. doi:10.1023/A:1015208017674.

Olsen, E.M., Heino, M., Lilly, G.R., Morgan, M.J., Brattey, J., Ernande, B., and Dieckmann, U. 2004. Maturation trends indicative of rapid evolution preceded the collapse of northern cod. Nature (London), **428**(6986): 932–935. doi:10.1038/nature02430. PMID:15118724.

Ricker, W.E. 1954. Stock and recruitment. J. Fish. Res. Board Can. **11**: 559–623.

Rideout, R.M., and Morgan, M.J. 2007. Major changes in fecundity and the effect on population egg production for three species of Northwest Atlantic flatfish. J. Fish Biol. **70**(6): 1759–1779. doi:10.1111/j.1095-8649.2007.01448.x.

Rideout, R.M., and Morgan, M.J. 2009. Fecundity of cod in the northwest Atlantic. CSAS Res. Doc. 2009/020.

Rijnsdorp, A.D. 1993. Fisheries as a large-scale experiment on life-history evolution: disentangling phenotypic and genetic effects in changes in maturation and reproduction of North Sea plaice, *Pleuronectes platessa* L. Oecologia, **96**(3): 391–401. doi:10.1007/BF00317510.

Rijnsdorp, A.D. 1994. Population-regulating processes during the adult phase in flatfish. Neth. J. Sea Res. **32**(2): 207–223. doi:10.1016/0077-7579(94)90041-8.

Scott, B.E., Marteinsdottir, G., Begg, G.A., Wright, P.J., and Kjesbu, O.S. 2006. Effects of population size/age structure, condition and temporal dynamics of spawning on reproductive output in Atlantic cod (*Gadus morhua*). Ecol. Model. **191**(3–4): 383–415. doi:10.1016/j.ecolmodel.2005.05.015.

Shepherd, J., Pope, J.G., and Cousens, R.D. 1984. Variations in fish stocks and hypotheses concerning their links with climate. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, **185**: 255–267.

Sissenwine, M.P., and Shepherd, J.G. 1987. An alternative perspective on recruitment overfishing and biological reference points. Can. J. Fish. Aquat. Sci. **44**(4): 913–918. doi:10.1139/f87-110.

Trippel, E.A. 1999. Estimation of stock reproductive potential: history and challenges for Canadian Atlantic gadoid stock assessments. J. Northwest Atl. Fish. Sci. **25**: 61–81. doi:10.2960/J.v25.a6.

Walters, C.J., and Ludwig, D. 1981. Effects of measurement errors on the assessment of stock-recruitment relationships. Can. J. Fish. Aquat. Sci. **38**(6): 704–710. doi:10.1139/f81-093.

Yoneda, M., and Wright, P.J. 2004. Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. Mar. Ecol. Prog. Ser. **276**: 237–248. doi:10.3354/meps276237.

Application of a method for estimating effective population size and admixture using diagnostic single nucleotide polymorphisms (SNPs): implications for conservation of threatened Paiute cutthroat trout (*Oncorhynchus clarkii seleniris*) in Silver King Creek, California

Amanda J. Finger, Eric C. Anderson, Molly R. Stephens, and Bernard P. May

Abstract: The threatened Paiute cutthroat trout (*Oncorhynchus clarkii seleniris*, PCT) is endemic to Silver King Creek, California, USA, which was stocked with non-native trout beginning in 1930. Single nucleotide polymorphism (SNP) and microsatellite data reveal that the trout population in Silver King Creek is weakly structured and composed of introgressed California golden trout (*Oncorhynchus mykiss aguabonita*, CAGT), hatchery rainbow trout (*Oncorhynchus mykiss*, RT), and some native PCT. Two SNP groups were analyzed: (*i*) one mitochondrial and five autosomal SNPs, diagnostic between Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) or PCT and CAGT or RT and (*ii*) one mitochondrial and five autosomal SNPs nearly diagnostic between CAGT and RT. The five autosomal cutthroat–rainbow SNPs were used to jointly estimate the cutthroat trout mixing proportion in Silver King Creek and effective population size (N_e) of the admixed population, using a coalescent-based maximum likelihood method. Given the stocking history of Silver King Creek, there are two different scenarios that bound the range of expected point estimates for N_e. We obtain point estimates of N_e = 150 and N_e = 750 for Silver King Creek under these two scenarios. This method will be useful in cases with differentiated taxa and in prioritizing conservation and restoration programs where the populations of concern are introgressed.

Résumé : La truite fardée de Paiute (*Oncorhynchus clarkii seleniris*, PCT), une forme menacée, est endémique à Silver King Creek, Californie, É.-U., qui a été empoissonné de truites non indigènes depuis 1930. Des analyses de polymorphisme mononucléotidique (SNP) et des données de microsatellites révèlent que la population de truites de Silver King Creek possède une structure faible et comprend des truites dorées de Californie (*Oncorhynchus mykiss aguabonita*, CAGT) introgressées, des truites arc-en-ciel (*Oncorhynchus mykiss*, RT) de pisciculture et quelques PCT indigènes. Nous avons analysé deux groupes de SNP : (*i*) un SNP mitochondrien et cinq SNP autosomaux diagnostiques entre la truite fardée de Lahontan ou PCT et CAGT ou RT et (*ii*) un SNP mitochondrien et cinq SNP autosomaux presque diagnostiques entre CAGT et RT. Nous avons utilisé les cinq SNP autosomaux fardée–arc-en-ciel pour estimer conjointement la proportion de mélange de la truite fardée dans Silver King Creek et le N_e de la population mixte à l'aide d'une méthode de vraisemblance maximale basée sur la coalescence. Étant donné l'histoire de l'empoissonnement dans Silver King Creek, il y a deux scénarios différents d'établissement des limites des points d'estimation attendus de N_e. Nous obtenons des points d'estimation de N_e = 150 et de N_e = 750 pour Silver King Creek selon les deux scénarios. Cette méthode pourra s'avérer utile dans les cas où les taxons sont différenciés et dans l'établissement de priorités dans les programmes de conservation et de restauration lorsqu'il y a introgression des populations concernées.

[Traduit par la Rédaction]

Introduction

Hybridization of native populations with introduced species is a major concern for the conservation and management of threatened species (e.g., Rhymer and Simberloff 1996; Epifanio and Nielsen 2000; Allendorf et al. 2004) and can result in negative consequences, including outbreeding depression,

Received 17 June 2010. Accepted 26 March 2011. Published at www.nrcresearchpress.com/cjfas on 10 August 2011.
J21880

Paper handled by Associate Editor Eric Taylor.

A.J. Finger, M.R. Stephens, and B.P. May. Genomic Variation Lab, Department of Animal Science, University of California Davis, One Shields Avenue, Davis, CA 95616, USA.
E.C. Anderson. Fisheries Ecology Division, Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, 110 Shaffer Road, Santa Cruz, CA 95060, USA.

Corresponding author: A.J. Finger (e-mail: ajfinger@ucdavis.edu).

Can. J. Fish. Aquat. Sci. **68**: 1369–1386 (2011) doi:10.1139/F2011-075 Published by NRC Research Press

or reduced population fitness through the loss of co-adapted gene complexes and ecological adaptations (Templeton 1986; Rhymer and Simberloff 1996; Allendorf et al. 2001) and even genomic extinction (Rhymer and Simberloff 1996; Allendorf et al. 2004). To mitigate these consequences, managers should know the genetic identity of populations of interest before undertaking conservation or restoration work on introgressed populations.

Detecting and quantifying introgression is critical to effective management. However, historical records are often incomplete or nonexistent, anecdotal evidence is subject to bias, and morphological data can often be misleading (Allendorf and Leary 1988; Baumsteiger et al. 2005; Metcalf et al. 2007). Genetic markers can provide accurate information, not only of recent introgression, but for additional management-relevant metrics such as effective population size (N_e), evolutionary potential, and genetic variation. N_e is of conservation importance because it allows managers to know how quickly genetic drift and inbreeding are removing genetic diversity from a population. Therefore, it is an important metric to consider when choosing populations for restoration or reintroduction purposes. In addition, knowing N_e changes over time may illuminate how management strategies affect N_e.

Microsatellite loci, with their high variability and mutation rate, have been used extensively to study population structure and hybridization (e.g., Cordes et al. 2006). More recently, SNPs (single nucleotide polymorphisms) have also been used to analyze levels of genetic variation in populations (see Morin et al. 2009 and citations therein) as well to detect hybridization in several taxa, including salmonids (Stephens et al. 2009), plants (Mercure and Bruneau 2008), birds (willow warblers (*Phylloscopus trochilus*), Bensch et al. 2002), and voles (*Microtus* spp.; Belfiore et al. 2003). Relative to microsatellites, SNPs are more readily standardized across platforms, have a better understood mutation model, and have less potential for homoplasy (e.g. Morin et al. 2009). Further, SNP panels can be chosen to be diagnostic between groups of interest, given adequate taxonomic and therefore sequence divergence. Finally, it can be reasonably assumed that an SNP that is fixed in a species today was also fixed for that species within a certain number of generations in the past and future, allowing estimation of N_e and admixture proportions using a temporal method when only current samples can be obtained.

Introgressive hybridization with introduced non-native rainbow trout (*Oncorhynchus mykiss* ssp., herein RT) has played a major role in the decline of all subspecies of native cutthroat trout (*Oncorhynchus clarkii* ssp., herein CT) in western North America (e.g., Allendorf and Leary 1988; Trotter 2008). There are 14 subspecies of CT in North America, two of which went extinct in the 20th century (Behnke 1992). Five are listed as threatened under the Endangered Species Act and seven have been petitioned for listing (Muhlfeld et al. 2009*a*). Indeed, hybridization is the greatest threat to the threatened Paiute cutthroat trout (*Oncorhynchus clarkii seleniris*, herein PCT; US Fish and Wildlife Service 1975). The scenario of the extirpation of the PCT in its limited native range within the Silver King Creek watershed, California, is common among species and subspecies endemic to narrow ranges. The entire native range of the PCT was stocked with other closely related trout species, including California golden trout (*Oncorhynchus mykiss aguabonita*, herein CAGT) and hatchery RT, leading to its extirpation through introgression and competition with non-natives. Out-of-basin native populations persist only because PCT were transferred to fishless waters prior to the putative initial non-native fish stocking events.

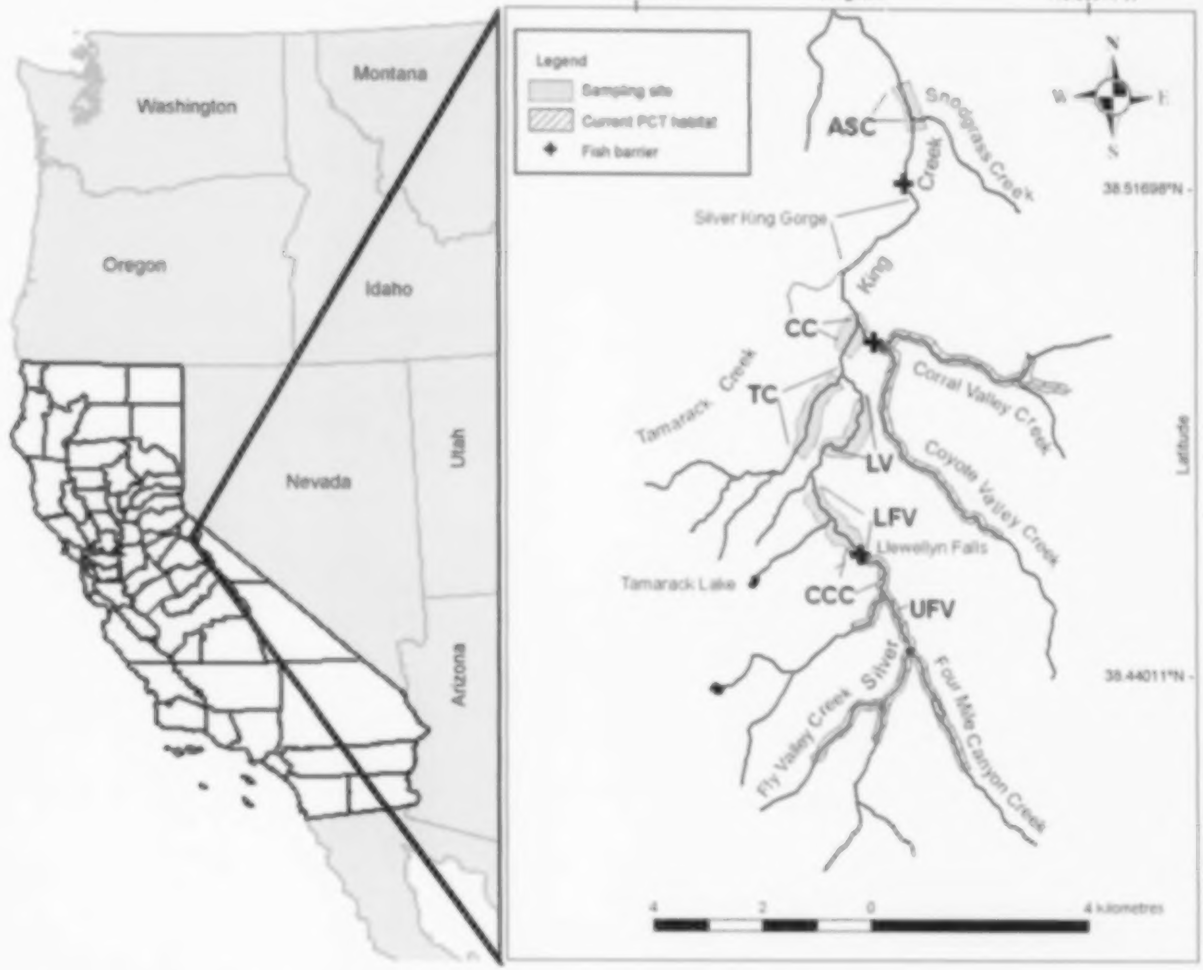
Finger et al. (2009) developed diagnostic SNP markers that distinguish PCT and the closely related Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*, herein LCT; together referred to as P/LCT) from RT for the purpose of quantifying introgression between the groups. We apply these and other markers to the trout population in Silver King Creek to accomplish three objectives: (*i*) evaluate the relative proportions of CAGT, RT, and P/LCT alleles in the population of fish in Silver King Creek today; (*ii*) describe a novel method that uses fixed SNP markers to simultaneously determine admixture proportions for and estimate N_e of an admixed population over time; and (*iii*) apply this method to the admixed population in Silver King Creek over a range of possible values.

Materials and methods

Study area overview and historical perspective

An approximately 10 km length of Silver King Creek, with the accessible reaches of three small tributaries (Tamarack Creek, Tamarack Lake Creek, and Coyote Valley Creek), constitutes the entire native habitat of the PCT (Fig. 1; Busack and Gall 1981; Cordes et al. 2004). Silver King Creek is a headwater tributary located in a high alpine meadow at 2500 m above sea level in the Carson–Iceberg Wilderness in Alpine County, California, that empties into the east fork of the Carson River. The upstream portion of this section of Silver King Creek is bounded by Llewellyn Falls, and the downstream portion is bounded by Silver King Gorge. These boundaries both act as fish barriers and are thought to have isolated PCT from LCT ~10 000 years ago. PCT generally has no body spots but may have up to nine spots on the body, while LCT is more heavily spotted (Behnke 1965). Between 1930 and 1994, Silver King Creek and Tamarack Lake, a small headwater lake connected to Silver King Creek, were planted with hatchery RT, CAGT, eastern brook trout (*Salvelinus fontinalis*), LCT, and PCT (which may have been introgressed with LCT; Table 1; W. Somer, California Department of Fish and Game, 1 Shields Avenue, Davis, California, personal communication, 2009). All of these non-native fish, except eastern brook trout, are known to hybridize with PCT (Busack and Gall 1981). Multiple reintroductions and efforts to eradicate hybrid fish from portions of Silver King Creek in the 1990s (see Cordes et al. 2004 for overview) culminated in a chemical treatment and restocking of Upper Silver King Creek above Llewellyn Falls in 1991–1993 from populations above fish barriers in the Silver King Creek watershed: Fly Valley Creek and Coyote Valley Creek (Fig. 1). Refuge populations of PCT are now restricted to portions of Silver King Creek in formerly fishless waters above fish barriers (Upper Silver King Creek above Llewellyn Falls, Coyote Valley Creek, Corral Valley Creek, Four Mile Canyon Creek, and Fly Valley Creek) and four out-of-basin locations (Cabin Creek and North Fork Cottonwood Creek in Mono County; Stairway Creek in Madera County; Sharktooth Creek in Fresno County). These refugial PCT populations were determined to

Fig. 1. Location of Silver King Creek in California and map of Silver King Creek between Silver King Gorge and headwaters. Sample locations are shaded in grey, and Paiute cutthroat trout (PCT) populations are shaded in grey with hatch marks. Fish barriers are denoted by a plus (+) symbol. Historic native PCT habitat was from Llewellyn Falls to Silver King Gorge. Abbreviations for hybrid fish in main stem Silver King Creek are as follows: ASC, Silver King Creek above Snodgrass Creek; CC, Canyon sites 1 and 2; TC, Tamarack Creek; LV, Long Valley; and LFV, Lower Fish Valley. Abbreviations for pure PCT above Llewellyn Falls are as follows: CCC, Connell's Cow Camp; and UFV, Upper Fish Valley.


Washington
Montana
Oregon
Idaho
Nevada
Utah
Arizona
119.62747°W
Longitude
119.58314°W
Legend
Sampling site
Current PCT habitat
Fish barrier
N
W
E
S
ASC
Snodgrass Creek
Creek
Silver King Gorge
King
38.51698°N
CC
Tamarack Creek
TC
Corral Valley Creek
LV
Coyote Valley Creek
Latitude
LFV
Llewellyn Falls
Tamarack Lake
CCC
UFV
Silver
Four Mile Canyon Creek
Fly Valley Creek
38.44011°N
4
2
0
4 kilometres

be non-introgressed with RT based on microsatellite data (Cordes et al. 2004). However, morphological evidence suggests that in the main stem portion of Silver King Creek below Llewellyn Falls, remaining P/LCT are introgressed with hatchery RT and CAGT (W. Somer, California Department of Fish and Game, personal communication, 2009). The Paiute Cutthroat Trout Recovery Plan (US Fish and Wildlife Service 2004) suggests the removal of hybrid fish and restocking with PCT in main stem Silver King Creek as part of restoring this species to its native range. Following this current study's genetic inventory of the non-native trout population in Silver King Creek, California Department of Fish and Game plans to chemically treat this reach, restock it with PCT, and adopt a monitoring program (W. Somer, California Department of Fish and Game, personal communication, 2009).

Sample collection and DNA extraction

To genetically characterize the trout population in main stem Silver King Creek, samples of hatchery RT, PCT, and CAGT populations were chosen as references based on presumed genetic similarity to source populations indicated by stocking records (Table 2). Samples from two hatcheries (Hot Creek strain (RT/HCS) and Mount Whitney strain (RT/MWS); collectively referred to as RT references) were selected to represent the RT stocked into Silver King Creek. For CAGT reference populations, fish from Volcano Creek (CAGT/VC), Cottonwood Lakes 2 (CAGT/CL2) and Cottonwood Lakes 4 (CAGT/CL4) were chosen (collectively referred to as CAGT references). CAGT/VC is non-introgressed based on microsatellite (Cordes et al. 2006) and SNP markers (Stephens 2007). Stocking records indicate that

Table 1. Known stocking history of Silver King Creek, with subspecies, years, recorded number of fish (N) stocked, and planting location.

Species	Year stocked	N	Location
Oncorhynchus mykiss (RT)	1930	5 000	Silver King Creek — below Lewellyn Falls
	1930	5 000	Silver King Creek — below Lewellyn Falls
	1931	10 000	Silver King Creek — below Lewellyn Falls
	1932	10 000	Silver King Creek — below Lewellyn Falls
	1933	10 000	Silver King Creek — below Lewellyn Falls
	1949	8 400	Silver King Creek — below Lewellyn Falls
	1949	5 040[a]	Silver King Creek — below Lewellyn Falls
	1951	6 010	Silver King Creek — below Lewellyn Falls
	1952	5 017	Silver King Creek — below Lewellyn Falls
	1953	4 960	Silver King Creek — below Lewellyn Falls
	1976	960	Silver King Creek — below Lewellyn Falls
	1976	2 900	Silver King Creek — below Lewellyn Falls
Oncorhynchus mykiss aguabonita (CAGT)	1969	1 018	Tamarack Lake
	1972	1 000	Tamarack Lake
	1973	1 141	Tamarack Lake
	1974	2 250	Tamarack Lake
	1976	2 272	Tamarack Lake
Oncorhynchus clarkii seleniris (PCT)	1985	173	Tamarack Lake
	1987	100	Tamarack Lake
	1991	Hundreds	Tamarack lake
Oncorhynchus clarkii henshawi (LCT)	1935	10 000	Silver King Creek — unknown location
	1946	8 700	Silver King Creek — unknown location
	1946	1 740	Silver King Creek – Poison Flat
	1946	1 740	Silver King Creek – Poison Flat
	1947	19 600	Coyote Creek — lower stream
	1947	19 600	Coyote Creek — lower stream
	1947	9 800	Tamarack Creek — lower stream
	1947	4 200	Tamarack Creek — lower stream
	1947	5 600	Silver King Creek – Forks[b]
	1955	1 005	Silver King Creek – Forks[b]
	1957	1 000	Silver King Creek – Forks — mouth
	1959	1 035	Silver King Creek – Forks — mouth
	1962	1 020	Silver King Creek – Forks[b]
	1967	4 000	Silver King Creek – Forks[b]
	1968	5 000	Coyote Creek — mouth to barrier
	1971	4 000	Coyote Creek — mouth to barrier
	1973	3 600	Tamarack Creek — above barrier
	1975	3 600	Tamarack Creek — above barrier
	1976	4 000	Tamarack Lake
	1980	4 200	Tamarack Lake
	1982	4 000	Tamarack Lake
	1987	3 000	Tamarack Lake
Salvelinus fontinalis (eastern brook trout)	1935	5 000	Silver King Creek — unknown location
	1968	500	Tamarack Lake

Note: RT, rainbow trout; CAGT, California golden trout; PCT, Paiute cutthroat trout; LCT, Lahontan cutthroat trout.

[a] Above Llewellyn Falls.

[b] Precise location of Forks is unknown — it is possibly the confluence of Silver King Creek with Coyote Valley Creek.

Cottonwood Lakes were the sources of CAGT planted in Silver King Creek; these populations are thought to be introgressed with hatchery RT (the date of introgression is uncertain, but may have been as early as the 1920s; Cordes et al. 2006). Finally, fish from the chemically treated and restored population in Upper Silver King Creek (from Upper Fish Valley (PCT/UFV) and Connell's Cow Camp (PCT/CCC); collectively referred to as PCT references) were chosen as PCT representatives; these fish were determined to be non-introgressed with RT or LCT, as determined by microsatellite (Cordes et al. 2004) and SNP analysis (Finger et al. 2009).

Fin clips were collected from 250 individual fish in Silver King Creek below Llewellyn Falls in 2006 (50 each from Lower Fish Valley (SKC/LFV), Long Valley (SKC/LV), Tamarack Creek (SKC/TC), Canyon Sites 1 and 2 (SKC/CC), and Silver King Creek above Snodgrass Creek (SKC/ASC); collectively referred to as SKC references). Thirty-

Table 2. Samples included in this study.

Subspecies – sample location	Site name	Abbreviation	Year	*N*
Silver King Creek	Lower Fish Valley	SKC/LFV	2006	50
	Long Valley	SKC/LV	2006	50
	Tamarack Creek	SKC/TC	2006	50
	Canyon Sites 1 and 2	SKC/CC	2006	50
	Silver King Creek above Snodgrass Creek	SKC/ASC	2006	50
Paiute cutthroat trout (*Oncorhynchus clarkii seleniris*)	Upper Fish Valley	PCT/UFV	2006	19
	Connell's Cow Camp	PCT/CCC	2006	19
California golden trout (*Oncorhynchus mykiss aguabonita*)	Volcano Creek	CAGT/VC	2001	34
	Cottonwood Lakes 2 (Lakes 1, 2, 3)	CAGT/CL2	2000	50
	Cottonwood Lakes 4 (Lakes 4, 5)	CAGT/CL4	2000	50
Rainbow trout (*Oncorhynchus mykiss irideus*)	Hot Creek strain, Hot Creek Hatchery	RT/HCS	2002	32
	Mt. Whitney Strain, Mt. Whitney Hatchery	RT/MWS	2002	30
Total				484

Note: Sampled individuals range from 1+ to 4 years old, with most individuals being 2+ years.

eight additional fish were sampled from Upper Silver King Creek (19 from PCT/UFV and 19 from PCT/CCC). To minimize relatedness among individuals sampled, ~10 fish for each sample site were collected at five locations (for up to ~50 individuals per site) within the site (see Fig. 1 for map of sites; shading and labels indicate sample location and total area of each site).

Whole genomic DNA was extracted from each fin clip using the Promega Wizard Extraction Kit and diluted to 5 ng·μL^{-1}. This DNA and DNA previously extracted from individuals from the reference populations were stored at –20 °C (Table 2 lists samples examined in this study).

SNP genotyping

A total of 12 TaqMan assays (Finger et al. 2009; Stephens et al. 2009) were performed on all reference populations listed (Table 2): one mitochondrial and five autosomal loci fixed for differences between P/LCT and *O. mykiss* ssp., (Table 3; B9_288, F5_136, HOXD_287, Rag11_280, URO_302 and Dloop_243; herein P/LCT SNPs); and one mitochondrial and five autosomal loci nearly fixed for differences between CAGT and RT (Table 4; A1A8_94, B9_388, ID1C_77–83, HOXD_170, RAG11_137, Dloop_316; herein CAGT SNPs). These SNPs were determined to be diagnostic or nearly diagnostic by surveying multiple populations of pure PCT, LCT, CAGT, and hatchery RT based on historical records, morphology, and previous genetic studies (see Finger et al. 2009 and Stephens et al. 2009 and citations therein). Though these markers are fixed or nearly fixed for a broad range of populations surveyed, the possibility exists that the subspecies or species of interest in this case may have shared alleles. Data for the six CAGT SNPs for CAGT/CL2, CAGT/CL4, RT/HCS, and RT/MWS were taken from Stephens (2007). TaqMan assays were run in Chromo4 Real-Time PCR Detector (MJ Research – Bio-Rad Laboratories, Inc., Hercules, California) in 96-well reaction plates. On each plate, four wells were reserved for positive and negative controls: one each with template from a known homozygote for each allele, one with DNA template from a known heterozygote, and one well with water as a negative control. MJ Opticon Monitor version 3.1.32 (MJ Research – BioRad Laboratories, Inc.) software was used to determine individual genotypes by creating a scatterplot depicting the endpoint fluorescence of each allele-specific probe in each well. Baseline average fluorescence over the 10- to 30-cycle range was subtracted to reduce noise, and endpoint fluorescence clusters were identified by comparing fluorescence with positive and negative controls.

SNP data analysis

Tests for significant pairwise linkage disequilibrium (LD) were implemented in Arlequin version 3.5 (Excoffier and Lischer 2010). Allele frequencies for each SNP marker were calculated and averaged over each sample location and population. Input files of autosomal SNP data were made for the software Structure version 2.2.3 (Pritchard et al. 2000) using the software Convert (Glaubitz 2004). Structure uses a Bayesian algorithm that, without prior information regarding each individual's membership to a population, computes by Markov chain Monte Carlo (MCMC) the posterior probability for the fraction, *q*, of each individual's ancestry that originates from each of *K* different genetic subgroups. The output of the MCMC from multiple runs at different values of *K* can be used to estimate the most likely *K*, the number of genetic clusters in the data. Two analyses were done with nuclear SNP data in Structure: (*i*) an analysis with the five autosomal P/LCT SNPs and the five autosomal CAGT SNPs for individuals from PCT, SKC, CAGT, and RT references with three runs each of *K* values 1–8; and (*ii*) an analysis with the five autosomal CAGT SNPs for SKC, CAGT, and RT references with three runs each of *K* values 1–5. Both analyses used the admixture model and had a burn-in period of 50 000 and 500 000 iterations. Clumpp version 1.1.2 (Jakobsson and Rosenberg 2007) was used to align multiple runs at each *K* value. The most likely estimated *K* value was determined using the method in Evanno et al. (2005). The software Distruct version 1.1 (Rosenberg 2004) was used to create a visual representation of the Structure output at the most likley *K* value.

Estimation of admixture proportions and N_e

We developed a simple, likelihood-based method for jointly estimating the admixture proportions and N_e of an admixed population with species- or subspecies-diagnostic SNP markers. Intuitively, the fraction of gene copies from each species or subspecies in the admixed population provides in-

Table 3. Paiute or Lahontan cutthroat trout (P/LCT) allele frequencies in Silver King Creek and reference populations at six P/LCT single nucleotide polymorphism loci.

Location	*N*	B9_228[a]	F5_136[a]	Dloop_243[a]	HOXD_287[a]	RAG11_280[a]	URO_302[a]	Avg.
PCT	38	1.0	1.0	1.0	1.0	1.0	1.0	1.0
SKC/LFV	50	0.0	0.0	0.0	0.0	0.01	0.0	<0.01
SKC/LV	50	0.01	0.0	0.0	0.02	0.01	0.0	0.01
SKC/TC	50	0.0	0.0	0.0	0.01	0.0	0.0	<0.01
SKC/CC	50	0.02	0.0	0.0	0.04	0.01	0.0	0.01
SKC/ASC	50	0.05	0.0	0.0	0.06	0.01	0.0	0.02
SKC total	250	0.02	0.0	0.0	0.03	0.01	0.0	0.01
CAGT/VC	34	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CAGT/CL2	46	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CAGT/CL4	46	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RT/HCS	30	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RT/MWS	49	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Note: Refer to Table 2 for location definitions.

[a] Finger et al. 2009.

formation about the admixture proportions, and the variation of that fraction from locus to locus provides information about the degree of genetic drift that has occurred in the population since the admixture event. The latter provides an estimate of N_e of the admixed population, if the time since admixture is known. We formalize this intuitive understanding of our procedure with a likelihood model based on the neutral coalescent (Kingman 1982), which automatically accounts for allele frequency variance due both to genetic drift and finite sampling.

The problem of jointly inferring admixture fractions and N_e of an admixed population was first addressed by Long (1991), who adopted a method-of-moments procedure. More recently several likelihood-based methods have been developed for the joint inference of admixture fractions and N_e. Chikhi et al. (2001) proposed a simple admixture model and provided a likelihood value in terms of the coalescent process. However, using MCMC, their method requires from days to weeks to run. Wang (2003) extended the underlying admixture model of Chikhi et al. (2001) to include an unobserved population from which both populations contributing to the admixture split in the past. He developed a pseudolikelihood for the model using the truncated matrix multiplication machinery introduced in Wang (2001). Wang (2006) described a coalescent-based likelihood suitable for sequence data that is applicable to the model proposed in Wang (2003).

The method we present is based on a simplified version of the admixture model in Chikhi et al. (2001). The likelihood for the model is based on the coalescent process, but, by exploiting the importance-sampling techniques introduced in Anderson (2005), the likelihood can be computed in a matter of seconds. This new method makes assumptions that are suitable only when using species- or subspecies-diagnostic SNPs. In such cases, however, unlike existing methods, our method can rapidly compute the entire joint likelihood surface for the admixture proportion and N_e (details appear in Appendix A). We estimated the N_e of the admixed Silver King Creek population using five autosomal, species-diagnostic P/LCT SNPs (listed in Table 3).

It is likely that the actual history of admixture between P/LCT and RT in Silver King Creek is considerably more complex than the single admixture event of our likelihood model. Given more certainty about the exact history of individual admixture events, we would be inclined to model the entire admixture history for the estimation of N_e. However, though stocking records are available (Table 1), they do not constitute a reliable reconstruction of the history of admixture, and many possible scenarios are possible, depending upon the success of the various introductions. For example, on one extreme, if the only successful introductions of any fish to Silver King Creek were those in 1930, then all the RT/CAGT alleles in Silver King Creek today would have descended from those 10 000 fish introduced in 1930, the PCT alleles would have descended from native fish, and the admixed population would have been undergoing genetic drift for 76 years (1930 to 2006) or roughly 25.3 generations (the average generation time for PCT is thought to be about 3 years; Wong 1975; the average generation time of rainbow trout is 2–3 years; Gall et al. 1988). At the other extreme, it is presumably possible that PCT in Silver King Creek were completely displaced by *O. mykiss* ssp. introductions between 1930 and 1976 and that the P/LCT alleles observed in the creek today originate from the 1991 stocking of PCT planted into Tamarack Lake. Under the latter scenario, drift in the admixed population would have been ongoing for only 15 years (1991 to 2006) or roughly five generations prior to sampling. We provide theory (see Supplemental Materials online[1]) to demonstrate that these two extreme admixture scenarios bound the degree of interlocus allele frequency variance expected in the admixed population and, hence, the possible estimates of N_e. The former scenario leads to an estimate of N_e about five times larger than the latter, since the latter scenario

---

[1] Supplementary data are available with the article through the journal Web site (http://www.nrcresearchpress.com/cjfas).

Table 4. California golden trout (CAGT) allele frequencies in Silver King Creek and reference populations at six CAGT SNP loci.

Location	A1A8_94[a]	B9_388[a]	ID1C_gap[a]	HOXD_170[b]	RAG11_137[a]	Dloop_316[a]	Avg.
PCT	0.0	1.0	0.0	1.0	—	1.0	—
SKC/LFV	0.10	0.55	0.17	0.41	0.21	0.02	0.24
SKC/LV	0.15	0.47	0.26	0.38	0.18	0.04	0.25
SKC/TC	0.01	0.41	0.04	0.37	0.10	0.0	0.16
SKC/CC	0.08	0.46	0.09	0.37	0.19	0.02	0.20
SKC/ASC	0.06	0.53	0.04	0.42	0.03	0.02	0.18
SKC total	0.08	0.50	0.12	0.39	0.14	0.02	0.20
CAGT/VC	1.0	0.94	1.0	1.0	1.0	1.0	0.99[a]
CAGT/CL2	0.94	0.93	0.92	0.99	0.89	0.87	0.92[c]
CAGT/CL4	0.82	0.94	0.90	0.92	0.93	1.0	0.92[c]
RT/HCS	0.0	0.07	0.0	0.18	0.0	0.0	0.04[c]
RT/MWS	0.0	0.02	0.0	0.02	0.02	0.0	0.01[c]

Note: Refer to Table 2 for location definitions.

[a] A1A8_94, B9_388, RAG11_137, and Dloop_316 and data for CAGT SNPs for the CAGT/VC population are from Stephens et al. (2009).

[b] Sprowles et al. (2006).

[c] Minor allele frequencies for the CAGT SNPs for populations CAGT/CL2, CAGT/CL4, RT/HCS, and RT/MWS are from Stephens (2007).

has about one fifth of the time for the observed degree of interlocus allele frequency variation to occur via drift. Results are given for both of these extreme scenarios.

Microsatellite genotyping

A total of 19 microsatellite loci (Table 5) were amplified in six multiplexed polymerase chain reactions in samples from 11 reference locations (listed in Table 1). We excluded PCT from microsatellite genotyping becazuse of poor amplification. Each reaction had a general master mix with a total volume of 10 μL with 2 ng template DNA, 2 mmol·L^{-1} $MgCl_2$, 125 μmol·L^{-1} each dNTP, and 0.1–0.2 μmol·L^{-1} each forward sequencing primer labeled with a fluorescent dye (either VIC, 6FAM, or NED), 0.1–0.2 μmol·L^{-1} each reverse primer, and 2 U *Taq* polymerase (Table 5). Reactions spent 4 min at 95 °C; 25 cycles of 30 s at 95 °C, 30 s at 58 °C, 45 s at 72 °C, followed by 45 min at 60 °C.

For each individual, a total volume of 10 μL consisting of 1.0 μL of each multiplexed PCR product diluted to 1:5 concentration with water, 0.2 μL LIZ600 size standard, and 8.8 μL formamide was placed in an individual well on a 96-well reaction plate. After being shocked at 95 °C for 3 min, the product was run on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, California). Resulting electropherograms were analyzed with Genemapper version 4.0 software (Applied Biosystems), and allelic sizes were confirmed manually. Individuals were rerun a maximum of two times for each failed locus, with each rerun containing previously genotyped controls. Individuals with <90% genotyping rate were not included in further analysis.

Microsatellite data analysis

Files were composed for the software programs Genepop (Raymond and Rousset 1995), Genetic Data Analysis (GDA; Lewis and Zaykin 2001), and Structure using the software program Convert (Glaubitz 2004). GDA was used to calculate observed (H_o) and expected (H_e) heterozygosities and number of alleles per locus for each population. Genepop was used to detect deviation from Hardy–Weinberg equilibrium for each locus for each population using the probability test. Genepop was also used to detect significant LD for each population and to calculate pairwise θ_{ST} values (Weir and Cockerham 1984). For both Hardy–Weinberg and LD tests, Markov chain parameters were 1000 dememorizations, 100 batches, and 1000 iterations per batch. Sequential Bonferroni corrections were used to calculate significance for multiple comparisons (Rice 1989). Six loci were dropped from further analysis because of low genotyping rates, unscorable alleles, or significant LD. A two-dimensional factorial correspondence analysis (FCA) was performed in Genetix version 4.05 (Belkhir et al. 1996–2004) to depict the relationships between all genotyped individuals. In FCA, two axes are created that are a composite of variables that optimize the differences between individuals. Individuals are then visualized as points along these axes.

As with SNP data, Structure version 2.2.3 was used to estimate the number of genetic clusters (K) among the RT, CAGT, and SKC references. Three runs each were done for K = 1–9 with the admixture model, a burn-in period of 100 000 and 1 000 000 iterations. The estimated K value was obtained as with the SNP data.

Results

SNP genotyping and analysis

The reference PCT fish are fixed for the P/LCT alleles at all six P/LCT SNP loci, and the RT reference fish are fixed for RT alleles at all six CAGT SNP loci. Out of 50 tests for SKC reference locations, none had significant LD ($p > 0.05$). SKC reference fish have very few P/LCT alleles (an average of 0.0 to 0.03; Table 3) at the P/LCT SNP loci. Of the fish with P/LCT alleles, all but one are heterozygous (one individual was homozygous for the P/LCT allele, *A*, at HOXD_287). No SKC reference fish have the P/LCT allele

Table 5. Multiplex primer combinations for microsatellite loci used including multiplex number (Multi), fluorescent label, reference, and primer concentration in multiplex reaction for forward (F) and reverse (R) primers.

Multi	Primer	Label	Primer reference	Primer concentration ($\mu mol \cdot L^{-1}$)
1	OMM1037	6FAM	Rexroad et al. 2002*a*	F, R = 0.1
	OMM1036	NED	Rexroad et al. 2002*a*	F, R = 0.1
	OMM1089	VIC	Rexroad et al. 2002*b*	F, R = 0.1
2	OtsG85	6FAM	Williamson et al. 2002	F, R = 0.2
	OMM1322	NED	Palti et al. 2002	F, R = 0.1
	OtsG423	VIC	Williamson et al. 2002	F, R = 0.1
3	OMM1082	6FAM	Rexroad et al. 2002*b*	F, R = 0.1
	Omy1009UW	NED	Spies et al. 2005	F, R = 0.1
	OMM1097	VIC	Rexroad et al. 2002*b*	F = 0.1, R = 0.2
4	OMM1046	NED	Rexroad et al. 2002*a*	F, R = 0.1
	OMM1078	VIC	Rexroad et al. 2002*b*	F, R = 0.1
	OMM1051	NED	Rexroad et al. 2002*a*	F, R = 0.1
	OtsG249b	6FAM	Williamson et al. 2002	F, R = 0.1
5	OMM1088	6FAM	Rexroad et al. 2002*b*	F, R = 0.1
	Omy1011UW	NED	Spies et al. 2005	F, R = 0.1
	OMM1058	VIC	Rexroad et al. 2002*b*	F = 0.1, R = 0.2
6	OtsG3	6FAM	Williamson et al. 2002	F, R = 0.1
	OMM1083	NED	Rexroad et al. 2002*b*	F = 0.1, R = 0.2
	OMM1081	VIC	Rexroad et al. 2002*b*	F = 0.1, R = 0.2

at the mitochondrial P/LCT SNP (Dloop_243). The furthest downstream SKC reference location samples have a few more P/LCT alleles (SKC/ASC, between 0.0 and 0.06 across loci; SKC/CC, between 0.0 and 0.04 across loci) than upstream locations (SKC/LFV, directly below Llewellyn Falls, between 0.0 and 0.01 across loci).

Sixteen individuals from main stem Silver King Creek have complete RT genotypes at all of the 12 SNPs, but none have complete CAGT genotypes. The proportion of CAGT alleles varies widely across CAGT SNP loci (between 0.03 and 0.48; Table 4) in Silver King Creek. At the mitochondrial locus (Dloop_316), the proportion of CAGT alleles is 0.02. The CAGT/CL2 and CAGT/CL4 populations each have an average of 0.92 CAGT alleles. In contrast, CAGT/VC has an average of 0.99 CAGT alleles. This supports other evidence of RT introgression in the Cottonwood Lakes populations (Cordes et al. 2006). At the CAGT SNPs, the PCT samples amplified at every locus except Rag11_137, and the PCT reference fish are homozygous for either CAGT alleles (B9_388, HOXD_170, and Dloop_316) or RT alleles (A1A8_94, ID1C_gap). In both sets of SNPs, the B9 and HOXD loci have a higher proportion of the non-RT allele in Silver King Creek fish than in the other SNPs. PCT and CAGT share an allele at B9_388 and HoxD_170 (Finger et al. 2009). It is possible that this inflates the proportion of CAGT alleles in Silver King Creek at these loci, but it is doubtful given the low proportion of P/LCT detected by the P/LCT SNPs.

The most likely *K* value for the Structure analysis of 10 autosomal SNPs is $K = 3$ genetic subgroups. The first cluster consists of the PCT references (UFV and CCC), the second consists of SKC and RT references, and the third cluster is composed of CAGT references (Fig. 2). In Silver King Creek, several individuals have *q* values indicating membership to multiple clusters, reflecting the history of hybridization. For the second Structure analysis, with only five autosomal CAGT SNPs, $K = 2$. SKC and RT references form one cluster, and the CAGT references group together to form the second cluster. In this analysis, the RT introgression in CAGT/CL2 and CAGT/CL4 is apparent in some individuals that are inferred to have ancestry from both clusters (Fig. 3*a*).

Microsatellite genotyping and analysis

All individuals from the SKC, RT, and CAGT reference populations were genotyped; those with a genotyping rate less than 90% after two rerun attempts were discarded from further analysis, leaving a total of 421 individual genotypes. Out of 936 pairwise LD comparisons, 88 are significant for LD before a Bonferroni correction and four are significant for LD after the correction. Across all sample locations, the total number of alleles for each locus ranges from 12 (Omm1058) to 28 (OtsG85), and the number of alleles per locus ranges from 5.61 (CAGT/VC) to 11.9 (SKC/LFV) (Table 6). Before a Bonferroni correction, 18 tests were significant for Hardy–Weinberg disequilibrium ($p < 0.05$). After the Bonferroni correction, seven tests were significant ($p < 0.05$). The microsatellite locus Omy1009UW was discarded from further analysis because of significant Hardy–Weinberg disequilibrium in the five collections from Silver King Creek ($p < 0.01$). All other loci are in Hardy–Weinberg equilibrium after the sequential Bonferroni correction for all populations, with two exceptions: Omm1088 in SKC/LFV ($p < 0.01$) and Omm1046 in SKC/CC ($p < 0.01$; Table 6). H_o ranges from 0.58 in CAGT/VC to 0.82 in the SKC/ASC location (Table 7). In general, heterozygosity values in SKC reference locations are greater than those for the CAGT and RT references. At several loci, alleles are present in the SKC reference that are not present in any of the other reference populations, indicating either genetic drift or that the refer-

Fig. 2. Depiction of clustering results from Structure analysis with five autosomal P/LCT SNP markers and five autosomal CAGT SNP markers. The most likely *K* value is 3. The first cluster (black) is the PCT from above Llewellyn Falls (PCT/UFV and PCT/CC). The second cluster (white) consists of hybrid fish from Silver King Creek (SKC/LFV, SKC/LV, SKC/TC, SKC/CC, and SKC/ASC) and the RT reference populations (RT/HCS and RT/MWS). The third cluster (gray) includes CAGT reference populations (CAGT/VC, CAGT/CL2, and CAGT/CL4). Refer to Table 2 for location definitions.

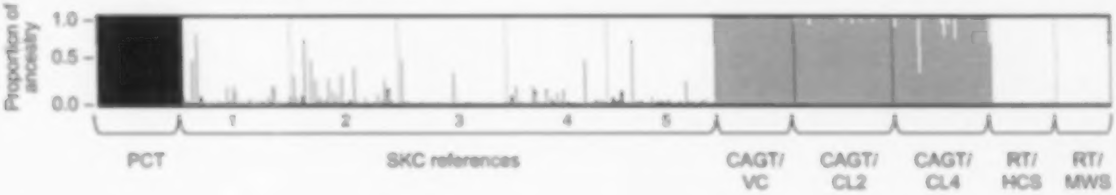
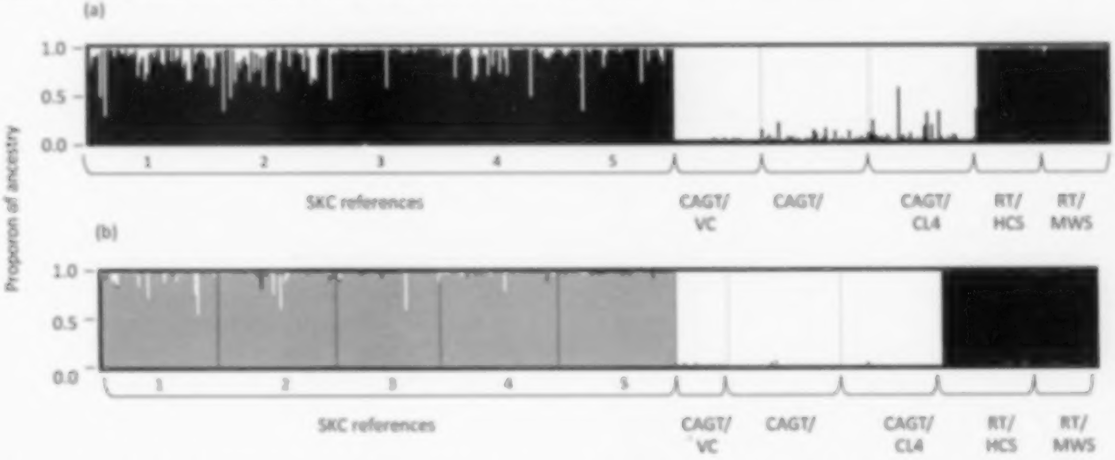


Fig. 3. (*a*, *b*) Depiction of clustering results from two Structure analyses. (*a*) Analysis with five autosomal CAGT SNPs. The most likely *K* value is 2. The first cluster (black) consists of hybrid Silver King Creek fish from five sample locations (1, Lower Fish Valley; 2, Long Valley; 3, Tamarack Creek; 4, Canyon Sites 1 and 2; and 5, Silver King Creek above Snodgrass Creek) and the RT reference populations (Hot Creek Strain (RT/HCS) and Mount Whitney Strain (RT/MWS)). The second cluster (white) includes the CAGT reference populations (Volcano Creek (CAGT/VC) and Cottonwood Lakes 2 and 4 (CAGT/CL2, CAGT/CL4)). (*b*) Depiction of clustering results from Structure analysis with 12 microsatellites including CAGT and RT references. The most likley *K* value is 3. The first cluster (gray) consists of the Silver King Creek fish from five sample locations (1, Lower Fish Valley; 2, Long Valley; 3, Tamarack Creek; 4, Canyon Sites 1 and 2; and 5, Silver King Creek above Snodgrass Creek). The second cluster (white) consists of CAGT reference populations (Volcano Creek (CAGT/VC) and Cottonwood Lakes 2 and 4 (CAGT/CL2, CAGT/CL4)). The third cluster (black) is the RT reference populations (Hot Creek Strain (RT/HCS) and Mount Whitney Strain (RT/MWS)). Refer to Table 2 for location definitions.



ence populations do not fully represent the true parental populations of trout in Silver King Creek.

Pairwise θ_{ST} values (summarized in Table 8) suggest that the population of trout in Silver King Creek is weakly structured (θ_{ST} values range from <0.01 to 0.04). Tamarack Creek (SKC/TC), a tributary to Silver King Creek, is slightly divergent from other SKC reference locations (θ_{ST} = 0.03–0.04), with a slightly higher proportion of RT alleles. Fish from CAGT/CL2 and CAGT/CL4 have a low pairwise θ_{ST} value (0.01) and are each moderately divergent from CAGT/VC (θ_{ST} = 0.20), which in turn is moderately divergent from all other populations (θ_{ST} = 0.20–0.30). This is probably due in part to low genetic diversity in CAGT/VC. In addition, CAGT/VC was the most divergent from RT references (θ_{ST} = 0.30). RT/MWS and RT/HCS are moderately divergent from each other (θ_{ST} = 0.19).

The FCA analysis produces a graph with four distinct clusters; the first consists of the SKC references, located near zero on both axes (Axis 1, 30.8% inertia; Axis 2, 23.1% inertia; Fig. 4). CAGT references group together at the negative end of Axis 2. RT/MWS fish form a cluster between the SKC reference cluster and a final cluster of fish from RT/HCS.

Structure analyses

The optimal *K* value for the Structure analysis is *K* = 3 for the microsatellite analyses. SKC reference fish form one cluster, CAGT references a second cluster, and RT references a third (Fig. 3*b*). In the microsatellite Structure analysis, in contrast with the SNP Structure analysis, SKC references form their own cluster, and the introgression with RT in CAGT/CL2 and CAGT/CL4 is less apparent.

Admixture and N_e

The maximum likelihood estimate (MLE) of the proportion of P/LCT ancestry in the admixed Silver King Creek population is 0.01, and the MLE of the effective size of this

Table 6. Results for 13 unlinked microsatellite loci analyzed in this study.

	OMM1037[a]	OMM1036[a]	OMM1089[b]	OtsG85[c]	OMM1322[d]	Omy1009UW[e]
No. of alleles per locus	15	18	25	28	23	27
Sample location (N)						
SKC/LFV (49)	0.67	0.17	0.58	0.21	0.37	**<0.01***
SKC/LV (50)	0.41	0.76	0.42	0.20	**0.02**	**<0.01***
SKC/TC (44)	0.17	0.44	0.10	0.58	**0.04**	**<0.01***
SKC/CC (50)	0.85	0.09	0.07	0.21	0.12	**<0.01***
SKC/ASC (50)	0.09	0.20	0.14	0.96	0.83	**<0.01***
CAGT/VC (22)	1.0	0.26	0.19	0.49	0.07	0.27
CAGT/CL2 (48)	0.53	0.30	0.92	0.20	0.45	0.18
CAGT/CL4 (43)	0.47	0.77	0.70	0.70	0.13	0.13
RT/HCS (31)	0.86	0.51	0.23	0.09	0.55	**0.05**
RT/MWS (34)	0.17	0.59	0.45	0.24	0.14	0.95

Note: Data include citations, total number of alleles per locus, and p values for Hardy–Weinberg test. Numbers in bold font are significant before a further analysis because of Hardy–Weinberg disequilibrium in the Silver King Creek references.

[a] Rexroad et al. 2002*a*.

[b] Rexroad et al. 2002*b*.

[c] Williamson et al. 2002.

[d] Palti et al. 2002.

[e] Spies et al. 2005.

Table 7. Results from genotyping 11 sample locations at 12 microsatellites.

Sample location	N	H_e	H_o	N_A
SKC/LFV	49	0.85	0.84	12.1
SKC/LV	50	0.84	0.82	12.4
SKC/TC	44	0.81	0.79	10.2
SKC/CC	50	0.84	0.83	12.6
SKC/ASC	50	0.84	0.86	12.1
CAGT/VC	22	0.59	0.58	5.61
CAGT/CL2	48	0.63	0.63	6.77
CAGT/CL4	43	0.62	0.63	6.61
RT/HCS	31	0.77	0.79	7.46
RT/MWS	34	0.74	0.70	7.38

Note: Data shown include sample number (N); expected (H_e) and observed (H_o) heterozygosity values; and mean number of alleles per locus per population (N_A).

population since the time of admixture under the $T = 25.3$ generation scenario is 750, and under the $T = 5$ generation scenario the MLE of N_e is 150. Likelihood surfaces with 95% confidence intervals for those two scenarios appear in Figs. 5*a* and 5*b*. The likelihood curves for N_e given each locus, individually, assuming an admixture fraction of 0.01, show that while there is considerable variation across loci, the MLE values (150 and 750, respectively) are within two units of log-likelihood of the maximum for every locus, suggesting that none of the loci are outliers and that the patterns of diversity can be explained by genetic drift alone (Figs. 5*c*, 5*d*).

Discussion

Genetic status of the trout in Silver King Creek

Based on SNP and microsatellite data, Silver King Creek is an admixed population of trout that is composed mostly of hatchery RT and CAGT. Indeed, despite being stocked with PCT and LCT for nearly 20 years after the last planting of *O. mykiss* ssp., the population in Silver King Creek now has very little P/LCT ancestry. Neither PCT nor LCT has successfully coexisted in Silver King Creek with CAGT or hatchery RT. This lack of success of cutthroat trout may be explained by several interacting factors, discussed in more detail below: stocking history in Silver King Creek (propagule pressure), the interactions between species in Silver King Creek, and the physical features of Silver King Creek. PCT were only stocked in Tamarack Lake, and the last time LCT was stocked in main stem Silver King Creek was 1957. Perhaps when P/LCT were stocked in Tamarack Lake, the propagule pressure from the continuous stocking of CAGT and RT from 1930 to 1976 allowed the establishment of a robust population of *O. mykiss* ssp. Subsequent stocking of P/LCT trout in Tamarack Lake was insufficient to establish substantial numbers of P/LCT genes in the main stem. Perhaps the habitat in Tamarack Lake is unsuitable (it is currently fishless) or the P/LCT did not move downstream. However, CAGT were also stocked in Tamarack Lake, and CAGT is genetically well represented in Silver King Creek; CAGT may move downstream more readily than P/LCT. Recent studies have shown that propagule pressure, source connectivity, and habitat quality are major biotic and abiotic factors influencing the spread of hybridization between nonnative RT and native cutthroat trout (e.g., Muhlfeld et al. 2009*c*; Bennett et al. 2010).

Species interactions may also play a role in the genetic composition of trout in Silver King Creek today. Planted P/LCT trout and their hybrids may generally be out-competed by CAGT and RT subspecies and their hybrids. This could be due to preferential mating or greater fitness of hybrids with more RT genes. Metcalf et al. (2008) surveyed hybrid populations of introduced RT and two subspecies of cutthroat trout native to Colorado and found a higher frequency of RT mtDNA than expected in hybrid populations, even in locations where there was more cutthroat trout nuclear DNA than RT nuclear DNA. This suggests asymmetrical pairings and the possibility of, in some cases, RT swamping out cutthroat genes. Our data provides some evidence of asymmetri-

OMM1046^{a}	OtsG249b^{f}	OMM1088^{b}	Omy1011UW^{e}	OMM1058^{b}	OtsG3^{c}	OMM1081^{b}
16	17	21	15	12	16	16
1.0	0.45	**<0.01***	0.18	0.76	0.08	0.54
0.08	0.51	0.29	**0.03**	0.05	0.67	0.77
0.02	0.07	**0.02**	0.08	0.19	0.07	0.28
<0.01*	**0.05**	0.05	0.80	0.25	0.37	0.31
0.41	0.58	0.07	0.86	0.24	0.97	0.59
0.16	0.06	0.24	1.0	1.0	—	0.88
0.27	0.75	**0.04**	0.44	1.0	1.0	0.10
0.91	0.94	0.31	0.16	1.0	0.11	0.87
0.08	0.12	**0.02**	**0.04**	0.13	0.41	0.50
0.88	0.14	**0.03**	0.29	0.08	0.71	0.13

Bonferroni correction; numbers with an asterisk (*) are significant after Bonferroni correction. Omy1009UW was discarded before

cal hybridization patterns in Silver King Creek. The P/LCT allele was not present at the Dloop_243 locus, and only 0.02 of the alleles at Dloop_316 were of CAGT ancestry, suggesting lower reproductive success for pairings of CAGT or P/LCT females and male RT than the reciprocal mating. Additionally, behavioral factors such as spawn timing and location or straying rates may make introgression between some taxa more likely than between others.

Physical features of the stream system such as fish barriers can also affect the distribution of alleles (e.g., Rubidge and Taylor 2005, but see Rasmussen et al. 2010). It appears that little gene flow is occurring over Llewellyn Falls into main stem Silver King Creek from the upstream PCT populations. In addition, the two furthest downstream Silver King Creek locations are separated from upstream populations of non-introgressed PCT (Coyote Valley Creek and Corral Valley Creek) by fish barriers of similar size to Llewellyn Falls. This genetic evidence, in addition to anecdotal evidence from 1991–1993 chemical treatments, supports the notion that PCT do not move much (W. Somer, California Department of Fish and Game, personal communication, 2009). It appears that many biotic and abiotic variables can affect the hybridization dynamics between trout species (e.g., Heath et al. 2010; Muhlfeld et al. 2009*c*).

California golden trout × *rainbow trout*

The hybridization dynamics between CAGT and RT are not as well studied as those between cutthroat trout and RT. In main stem Silver King Creek, though only stocked five times (in Tamarack Lake, between 1969 and 1976), CAGT persist genetically at surprisingly high percentages (0%–48% across SNP loci). Fish from the upstream portion of main stem Silver King Creek (SKC/LFV and SKC/LV) have a slightly higher proportion of CAGT alleles (an average of 0.24 for both locations). CAGT were only planted in Tamarack Lake and must have moved downstream from Tamarack Lake into the main stem, leaving a genetic signature in SKC/LFV and SKC/LV. The high proportion of CAGT in Silver King Creek relative to P/LCT suggests the possibility of hybrid vigor with RT compared with P/LCT × RT hybrids. This may be because CAGT and RT are more closely related to each other than they are to cutthroat trout, so their offspring have greater fitness (e.g., Behnke 1992; McKay et al. 1996). Alternatively, even if the F_2 offspring do not generally have higher fitness, F_1 hybrids may have very high fitness, or a few individuals with a high proportion of non-native admixture may have very high fitness and reproductive success leading to dominance of CAGT and RT.

N_e and admixture analysis

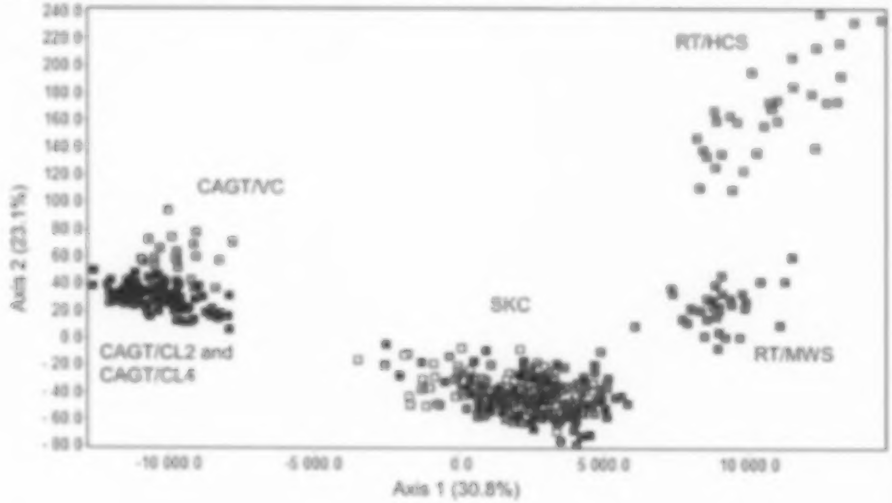
In jointly estimating admixture and N_e of the fish population in Silver King Creek, we used only the autosomal P/LCT SNPs, because the CAGT SNPs are not completely fixed. If species-specific markers were found for CAGT and RT, the method could be used to determine the admixed proportion of CAGT and provide an independent estimate of N_e. Because of uncertainty around the exact history of admixture, we analyzed the data under the two most extreme possible scenarios. The admixture event likely occurred at least three generations ago, but we need more data to be more precise. Given this caveat, the MLE of N_e in Silver King Creek based on the P/LCT SNPs is 150 under a scenario in which all CT alleles descend entirely from the most recent PCT planting (PCT in 1991). Under a different scenario in which all CT alleles descend exclusively from the original native PCT population, and the RT alleles were derived entirely from the 1930 planting of RT, the MLE of N_e is 750.

The census size (N_c) of all Silver King Creek fish below Llewellyn Falls, based on population data from 2000 (W. Somer, California Department of Fish and Game, personal communication, 2009), is estimated to be around 2000 fish. According to this estimation, the ratio of N_e/N_c is 0.08 and 0.38, respectively, under the two scenarios of MLEs of 150 and 750. These values are on the low and high ends of the range for vertebrate, invertebrate, and plant populations (Frankham 1995). In a review of contemporary N_e, all cate-

Table 8. Pairwise θ_{ST} values based on 12 microsatellites.

Location	SKC/LFV	SKC/LV	SKC/TC	SKC/CC	SKC/ASC	CAGT/VC	CAGT/CL2	CAGT/CL4	RT/HCS
SKC/LV	<0.01	—							
SKC/TC	0.04	0.03	—						
SKC/CC	<0.01	<0.01	0.03	—					
SKC/ASC	0.01	0.01	0.03	0.01	—				
CAGT/VC	0.20	0.21	0.24	0.23	0.22	—			
CAGT/CL2	0.16	0.16	0.20	0.18	0.20	0.21	—		
CAGT/CL4	0.17	0.17	0.21	0.19	0.20	0.23	0.01	—	
RT/HCS	0.15	0.15	0.15	0.14	0.15	0.30	0.28	0.29	—
RT/MWS	0.12	0.12	0.14	0.12	0.12	0.30	0.27	0.29	0.19

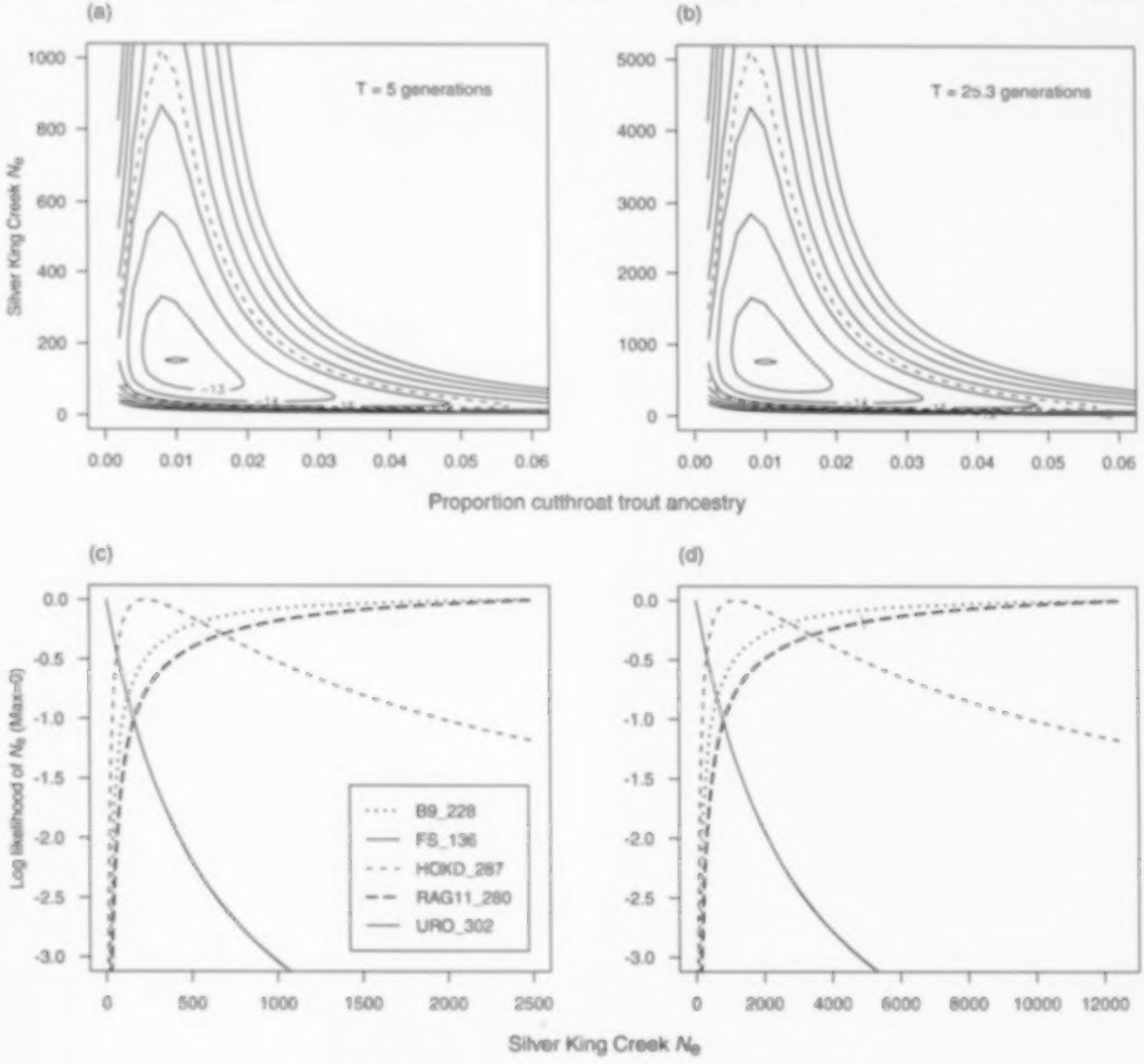
Fig. 4. Factorial correspondence analysis (FCA) analysis of samples genotyped with 12 microsatellites. Four distinct clusters are produced: (*i*) Silver King Creek (SKC) samples, consisting of Lower Fish Valley, Long Valley, Tamarack Creek, Canyon Sites 1 and 2, and SKC above Snodgrass Creek; (*ii*) California golden trout (CAGT) reference populations consisting of Cottonwood Lakes 2 and 4 (CAGT/CL2 and CAGT/CL4) and Volcano Creek (VC); (*iii*) Hot Creek Strain rainbow trout (RT/HCS); and (*iv*) Mount Whitney Strain rainbow trout (RT/MWS).



gories of populations (exploited, stable, and conservation) in all studies surveyed had an average N_e/N_c ratio of 0.19 ± 0.11 (Palstra and Ruzzante 2008). As our two estimates of N_e represent values from two bounding, extreme scenarios for the admixture history, it seems reasonable that they should be just within or slightly above the low and high endpoints of Palstra and Ruzzante's (2008) interval. It should be noted, however, that it is difficult to compare N_e to N_c with an estimate of N_c from only a single point in time. Unrecorded variation over time in N_c would likely drive N_e down, since the rate of inbreeding and allele frequency drift is strongly influenced by small values of population size; our estimate of N_e is an estimate of the harmonic mean of N_e over multiple generations. The harmonic mean of N_c over time would provide a better comparison with our estimated N_e than either a single estimate of N_c in time or the arithmetic mean of N_c over multiple generations (Kalinowski and Waples 2002).

Our method for jointly estimating N_e and the admixture proportion is, like many statistical–genetic methods, based on an idealized model that might be violated in practice. Two departures from the model that one might expect are (*i*) population structure or, equivalently, incomplete admixture of the two species; and (*ii*) non-neutrality of the genetic markers. We briefly consider the effects of each of these on our estimator. If the admixed population is structured so that some locales have more P/LCT than RT ancestry, for example, then the model assumption of independence between loci would be violated. The same would be true if mating between the species is assortative so that the "admixed population" includes a large number of individuals that are close to being pure P/LCT and others that are close to being pure RT. If the population is structured in this way, the first problem is that it is difficult to ensure that a representative sample has been drawn. Assuming, however, that a sample representative of the diversity in the structured population has been drawn, there are still two other effects that the population structure will have: (*i*) the model will overestimate the degree of precision it has in estimating the admixture proportion, because it assumes independence of allelic types between loci within individuals (which is not the case in a structured population); and (*ii*) the model will overestimate N_e because the correlation between alleles at different loci ensures that the interlocus variance in allele frequencies will be smaller than it would be if there were no LD induced by the population structure. Perhaps the simplest way to gain an intuitive under-

Fig. 5. (*a*, *b*) Contour plots of the joint likelihood surface for the admixture proportion of cutthroat trout in Silver King Creek and the N_e of the admixed population under two admixture history scenarios: (*a*) $T = 5$ and (*b*) $T = 25.3$ generations (see text). The dashed line shows the approximate 95% confidence interval. (*c*, *d*) Log-likelihood of N_e for individual loci assuming a cutthroat admixture fraction of 0.01. Values are scaled so the maximum of each curve is 0. (*c*) $T = 5$ generations scenario. (*d*) $T = 25.3$ generations. Of note, none of the curves show a log-likelihood difference greater than 2.0 from the maximum at the maximum likelihood estimate values of 150 and 750, respectively. The log-likelihood curves for FS_136 and URO_302 are entirely overlapping and indistinguishable; both follow the solid black line in the figure.



standing of this is by considering what would happen if every individual from the "admixed" population were either a pure PCT or pure RT. In that case, representative sampling will give an unbiased estimate of the mixing proportion of the two species, but the frequencies of the species-diagnostic alleles will be identical at every locus — the additional loci would provide no extra information for estimating the admixture fractions, and the interlocus variance in allele frequency will be zero, implying the N_e estimate should be infinite. The degree that population structure or assortative mating will bias the estimate of N_e is clearly determined by the correlation of allelic type within individuals. Since we found no evidence for significant LD between the five P/LCT SNPs in the Silver King Creek sample, we suspect that our results are not biased upward by this effect.

The assumptions of our model would also be violated if the SNPs used were linked to regions of the genome that were under selection in the admixed population. In that case, the allele frequencies at every locus would be affected by more than just genetic drift, and the inclusion of some loci under selection could lead to an increased interlocus allele frequency variance that would bias estimates of N_e downward. In fact, selection's tendency to increase heterogeneity between loci is at the basis of many tests for selection (Lewontin and Krakauer 1973; Beaumont and Balding 2004; Foll and Gaggiotti 2008). The five P/LCT SNPs we used do not show evidence of heterogeneity beyond that expected by genetic drift, which suggests that natural selection has not necessarily affected our estimate of N_e. Had we used more loci, however, it is possible that some could have been linked to regions subject to strong selection. We point out that the likelihood model developed here provides an appropriate null model for detecting outlier loci in admixed populations. As more species- and subspecies-diagnostic SNPs become available and are mapped to the genome, we predict that our method, being fast and computationally efficient, can be incorporated into new tests that use recently admixed populations to identify gene regions involved in hybrid incompatibility, fitness in different environments, and, possibly, speciation.

The relatively high N_e estimates of 150 to 750 fish suggest that genetic drift is only a weak factor in removing genetic diversity over time. These fish have abundant heterozygosity with which to adapt to their environment. Analysis of parental admixture proportions and N_e will be particularly useful in situations of conservation interest when possible source populations must be examined for conservation value and priority (Allendorf et al. 2001), such as when introgression levels of native CT are deemed low enough to be acceptable or when non-introgressed native CT populations no longer exist. In such instances, inexpensive and efficient detection of hybridization is necessary, and knowledge of the demographic and management history of a population is of interest. The application of this method using these or other diagnostic SNPs can facilitate the ranking of populations' conservation utility; those with higher N_e and higher genetic diversity, as well as higher proportion of, for example, native cutthroat trout ancestry, may be better suited for restoration, reintroduction, or translocation. The discovery of fixed diagnostic SNPs for other taxa will enable a wider use of the simultaneous estimation method in such conservation situations.

Management and conservation implications

The biodiversity of native trout in western North America is declining, in large part because of introgression with hatchery fish planted over the last century (e.g., Trotter 2008; Behnke 1992). The result of this widespread stocking is hundreds of hybrid populations and the reduced viability of native fishes. Moreover, managers have limited options when attempting to restore native trout populations in a location where introgression has occurred. Complete restoration involves two steps: (*i*) removal or control of the spread of introgressed fish and (*ii*) restocking with an appropriate source of native fish. For removal or control of introgression, in smaller areas chemical treatment to remove introgressed fish may be the best option, given that once introgression has begun it is nearly impossible to stop. Chemical treatment requires complete removal of all fish present and subsequent monitoring to ensure that treatment success. A second management option, often undertaken when there are legal, political, or practical obstacles to chemical treatment, is to stop stocking fish altogether and hope that the natives recover. For example, in Montana, stocking of non-native RT stopped in 1969; however, there is anecdotal evidence that 70 000 RT were illegally released from a hatchery in 1997 (Muhlfeld et al. 2009*b*), and introgression with native westslope cutthroat trout has continued. There are several factors that may influence this introgression, such as straying rates (Boyer et al. 2008); neighborhood effects (Hitt et al. 2003); and stream temperature, stocking history, and land-use disturbance (Muhlfeld et al. 2009*c*). Regardless of how introgression spreads, it is expected to continue until there are no pure parental types (Hitt et al. 2003).

How managers decide to tackle problems of introgression is context-dependent. In some situations the only native populations of trout may be hybrid populations, and the best option may be to conserve the populations with the lowest proportion of non-native admixture. In contrast, there may be situations where nonhybridized populations remain but are geographically distant. Even if there are hybrid populations in close proximity, managers may wish to suppress or eradicate any hybrid populations, even those with very low non-native admixture.

Diagnostic SNP markers provide an excellent tool for managing introgression and restocking, both of which require considerable time and resources. In the foreseeable future the cost of designing assays will likely decline and throughput will increase, and there will be hundreds of diagnostic markers available. Microsatellites and allozymes have been more standard tools for characterizing hybridization, but it is much more difficult to find diagnostic markers. In addition, in cases where there are only a few individuals left of a population or taxon, SNPs can correctly classify individuals.

After discontinuing the stocking of non-native fish and the removal of introgressed fish, managers must select an appropriate donor source population of native fish for restocking. Initial misidentification of greenback cutthroat trout (*Oncorhynchus clarkii stomias*) sources used for restocking and propagation efforts resulted in the reintroduction of fish introgressed with closely related Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*, a Colorado subspecies of special concern; Metcalf et al. 2007). In addition, those greenback populations that were indeed "pure" had very low genetic diversity, increasing the risk of inbreeding. This example underscores the need for correct genetic characterization of donor sources in conservation situations.

In the case of Silver King Creek, despite the limited stocking of PCT in an interconnected headwater lake and the termination of stocking *O. mykiss* ssp. in 1976, our results suggest that the PCT has not and will not likely recover. For the purpose of reintroducing PCT to Silver King Creek, there is little reason to preserve the current population of trout below Llewellyn Falls, given such few P/LCT alleles and the existence of several refuge populations of non-introgressed PCT. Achieving the goal of successful restoration of PCT will require total removal of the trout in Silver King Creek to prevent future hybridization. Genetic monitoring will play a key role in re-establishing a self-sustaining population of PCT, allowing for early detection of hybridization due to unauthorized stocking events or unsuccessful hybrid eradication.

Acknowledgements

The authors thank William Somer for extensive consultation and the map of Silver King Creek. In addition the authors thank the California Department of Fish and Game and the United States Fish and Wildlife Service for sampling and project support. Thanks also go to the Threatened Trout Crew for samples and Karrigan Bork for valuable comments and editing. Funding for this project was provided through a Section 6 grant for Paiute trout habitat restoration by the US Fish and Wildlife Service, contract No. P0882011 with California Department of Fish and Game.

References

Allendorf, F.W., and Leary, R.F. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conserv. Biol. **2**(2): 170–184. doi:10.1111/j.1523-1739.1988.tb00168.x.

Allendorf, F.W., Leary, R.F., Spruell, P., and Wenburg, J.K. 2001. The problems with hybrids: setting conservation guidelines. Trends Ecol. Evol. **16**(11): 613–622. doi:10.1016/S0169-5347(01)02290-X.

Allendorf, F.W., Leary, R.F., Hitt, N.P., Knudsen, K.L., Lundquist, L.L., and Spruell, P. 2004. Intercrosses and the U.S. endangered species act: Should hybridized populations be included as westslope cutthroat trout? Conserv. Biol. **18**(5): 1203–1213. doi:10.1111/j.1523-1739.2004.00305.x.

Anderson, E.C. 2005. An efficient Monte Carlo method for estimating N_e from temporally spaced samples using a coalescent-based likelihood. Genetics, **170**(2): 955–967. doi:10.1534/genetics.104.038349. PMID:15834143.

Beaumont, M.A., and Balding, D.J. 2004. Identifying adaptive genetic divergence among populations from genome scans. Mol. Ecol. **13**(4): 969–980. doi:10.1111/j.1365-294X.2004.02125.x. PMID:15012769.

Baumsteiger, J., Hankin, D., and Loudenslager, E.J. 2005. Genetic analyses of juvenile steelhead, coastal cutthroat trout, and their hybrids differ substantially from field identification. Trans. Am. Fish. Soc. **134**(4): 829–840. doi:10.1577/T04-114.1.

Behnke, R.J. 1965. A systematic study of the family Salmonidae with special reference to the genus *Salmo*. Ph.D. thesis, University of California, Berkeley, Calif.

Behnke, R.J. 1992. Native trout of western North America. American Fisheries Society, Bethesda, Md. AFS Monograph No. 6.

Belfiore, N.M., Hoffman, F.G., Baker, R.G., and Dewoody, J.A. 2003. The use of nuclear and mitochondrial single nucleotide polymorphisms to identify cryptic species. Mol. Ecol. **12**(7): 2011–2017. doi:10.1046/j.1365-294X.2003.01874.x. PMID:12803650.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. 1996–2004. GENETIX 4.05, logiciel sous Windows pour la génétique des populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Bennett, S.N., Olson, J.R., Kershner, J.L., and Corbett, P. 2010. Propagule pressure and stream characteristics influence introgression: cutthroat and rainbow trout in British Columbia. Ecol. Appl. **20**(1): 263–277. doi:10.1890/08-0441.1. PMID:20349846.

Bensch, S., Akesson, S., and Irwin, D. 2002. The use of AFLP to find an informative SNP: genetic differences across a migratory divide in willow warblers. Mol. Ecol. **11**(11): 2359–2366. doi:10.1046/j.1365-294X.2002.01629.x. PMID:12406246.

Boyer, M.C., Muhlfeld, C.C., and Allendorf, F.W. 2008. Rainbow trout (*Oncorhynchus mykiss)* invasion and the spread of hybridization with native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). Can. J. Fish. Aquat. Sci. **65**(4): 658–669. doi:10.1139/f08-001.

Busack, C.A., and Gall, G.A.E. 1981. Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*). Can. J. Fish. Aquat. Sci. **38**(8): 939–951. doi:10.1139/f81-127.

Chikhi, L., Bruford, M.W., and Beaumont, M.A. 2001. Estimation of admixture proportions: a likelihood-based approach using Markov chain Monte Carlo. Genetics, **158**(3): 1347–1362. PMID:11454781.

Cordes, J.F., Israel, J.A., and May, B. 2004. Conservation of Paiute cutthroat: the genetic legacy of population transplants in an endemic California salmonid. Calif. Fish Game, **90**(3): 101–118.

Cordes, J.F., Stephens, M.R., Blumberg, M.A., and May, B. 2006. Identifying introgressive hybridization in native populations of California golden trout based on molecular markers. Trans. Am. Fish. Soc. **135**(1): 110–128. doi:10.1577/T05-120.1.

Epifanio, J., and Nielsen, J. 2000. The role of hybridization in the distribution, conservation and management of aquatic species. Rev. Fish Biol. Fish. **10**(3): 245–251. doi:10.1023/A:1016729132297.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol. **14**(8): 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x. PMID:15969739.

Excoffier, L., and Lischer, H.E.L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetic analyses under Linux and Windows. Mol. Ecol. Resour. **10**(3): 564–567. doi:10.1111/j.1755-0998.2010.02847.x. PMID:21565059.

Finger, A.J., Stephens, M.R., Clipperton, N.W., and May, B. 2009. Six diagnostic single nucleotide polymorphism markers for detecting introgression between cutthroat and rainbow trouts. Mol. Ecol. Resour. **9**(3): 759–763. doi:10.1111/j.1755-0998.2009.02532.x. PMID:21564737.

Foll, M., and Gaggiotti, O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics, **180**(2): 977–993. doi:10.1534/genetics.108.092221. PMID:18780740.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genet. Res. **66**(2): 95–107. doi:10.1017/S0016672300034455. PMID:18976539.

Gall, G.A.E., Baltodano, J., and Huang, N. 1988. Heritability of age at spawning for rainbow trout. Aquaculture, **68**(2): 93–102. doi:10.1016/0044-8486(88)90233-5.

Glaubitz, J.C. 2004. CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol. Ecol. Notes, **4**(2): 309–310. doi:10.1111/j.1471-8286.2004.00597.x.

Heath, D., Bettles, C.M., and Roff, D. 2010. Environmental factors associated with reproductive barrier breakdown in sympatric trout populations on Vancouver Island. Evol. Appl. **3**(1): 77–90. doi:10.1111/j.1752-4571.2009.00100.x.

Hitt, N.P., Frissell, C.A., Muhlfeld, C.C., and Allendorf, F.W. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. Can. J. Fish. Aquat. Sci. **60**(12): 1440–1451. doi:10.1139/f03-125.

Jakobsson, M., and Rosenberg, N.A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, **23**(14): 1801–1806. doi:10.1093/bioinformatics/btm233. PMID:17485429.

Kalinowski, S.T., and Waples, R.S. 2002. Relationship of effective to census size in fluctuating populations. Conserv. Biol. **16**(1): 129–136. doi:10.1046/j.1523-1739.2002.00134.x.

Kingman, J.F.C. 1982. On the genealogy of large populations. J. Appl. Prob. **19**: 27–43. doi:10.2307/3213548.

Lewis, P.O., and Zaykin, D. 2001. GENETIC DATA ANALYSIS: a computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors, available from http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php.

Lewontin, R.C., and Krakauer, J. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics, **74**(1): 175–195. PMID:4711903.

Long, J.C. 1991. The genetic structure of admixed populations. Genetics, **127**(2): 417–428. PMID:2004712.

McKay, S.J., Devlin, R.H., and Smith, M.J. 1996. Phylogeny of Pacific salmon and trout based on growth hormone type-2 and mitochondrial NADH dehydrogenase subunit 3 DNA sequences. Can. J. Fish. Aquat. Sci. **53**(5): 1165–1176. doi:10.1139/f96-042.

Mercure, M., and Bruneau, A. 2008. Hybridization between the escaped *Rosa rugosa* (Rosaceae) and native *R. blanda* in eastern North America. Am. J. Bot. **95**(5): 597–607. doi:10.3732/ajb.2007385. PMID:21632386.

Metcalf, J.L., Pritchard, V.L., Silvestri, S.M., Jenkins, J.B., Wood, J.S., Cowley, D.E., Evans, P.R., Shiozawa, D.K., and Martin, A.P. 2007. Across the great divide: genetic forensics reveals misidentification of endangered cutthroat trout populations. Mol. Ecol. **16**(21): 4445–4454. doi:10.1111/j.1365-294X.2007.03472.x. PMID:17727621.

Metcalf, J.L., Siegle, M.R., and Martin, A.P. 2008. Hybridization dynamics between Colorado's native cutthroat trout and introduced rainbow trout. J. Hered. **99**(2): 149–156. doi:10.1093/jhered/esm118. PMID:18238784.

Morin, P.A., Martien, K.K., and Taylor, B.L. 2009. Assessing statistical power of SNPs for population structure and conservation studies. Mol. Ecol. Resour. **9**(1): 66–73. doi:10.1111/j.1755-0998.2008.02392.x. PMID:21564568.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., and Allendorf, F.W. 2009*a*. Hybridization rapidly reduces fitness of a native trout in the wild. Biol. Lett. **5**(3): 328–331. doi:10.1098/rsbl.2009.0033. PMID:19324629.

Muhlfeld, C.C., McMahon, T.E., and Belcer, D. 2009*b*. Spatial and temporal spawning dynamics of native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), introduced rainbow trout (*Oncorhynchus mykiss*), and their hybrids. Can. J. Fish. Aquat. Sci. **66**: 1153–1168. doi:10.1139/F09-073.

Muhlfeld, C.C., McMahon, T.E., Boyer, M.C., and Gresswell, R.E. 2009*c*. Local-habitat, watershed, and biotic factors in the spread of hybridization between native westslope cutthroat trout and introduced rainbow trout. Trans. Am. Fish. Soc. **138**(5): 1036–1051. doi:10.1577/T08-235.1.

Palstra, F.P., and Ruzzante, D.E. 2008. Genetic estimates of contemporary effective population size: What can they tell us about the importance of genetic stochasticity for wild population persistence? Mol. Ecol. **17**(15): 3428–3447. doi:10.1111/j.1365-294X.2008.03842.x. PMID:19160474.

Palti, Y., Fincham, M.R., and Rexroad, C.E. 2002. Characterization of 38 polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). Mol. Ecol. Notes, **2**(4): 449–452. doi:10.1046/j.1471-8286.2002.00274.x.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, **155**(2): 945–959. PMID:10835412.

Rasmussen, J.B., Robinson, M.D., and Heath, D.D. 2010. Ecological consequences of hybridization between native westslope cutthroat (*Oncorhynchus clarkii lewisi*) and introduced rainbow (*Oncorhynchus mykiss*) trout: effects on life history and habitat use. Can. J. Fish. Aquat. Sci. **67**(2): 357–370. doi:10.1139/F09-191.

Raymond, M., and Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. **86**(3): 248–249.

Rexroad, C.E., III, Coleman, R.L., Gustafson, A.L., Hershberger, W. K., and Killefer, J. 2002*a*. Development of rainbow trout microsatellite markers from repeat enriched libraries. Mar. Biotechnol. **4**(1): 12–16. doi:10.1007/s10126-001-0058-6. PMID:14961284.

Rexroad, C.E., III, Coleman, R.L., Hershberger, W.K., and Killefer, J. 2002*b*. Rapid communication: thirty-eight polymorphic microsatellite markers for mapping in rainbow trout. J. Anim. Sci. **80**(2): 541–542. PMID:11881939.

Rhymer, J.M., and Simberloff, D. 1996. Extinction by hybridization and introgression. Annu. Rev. Ecol. Syst. **27**(1): 83–109. doi:10.1146/annurev.ecolsys.27.1.83.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**(1): 223–225. doi:10.2307/2409177.

Rosenberg, N.A. 2004. Distruct: a program for the graphical display of population structure. Mol. Ecol. Notes, **4**(1): 137–138. (Also available from http://rosenberglab.bioinformatics.med.umich.edu/distruct.html [modified 19 September 2010].) doi:10.1046/j.1471-8286.2003.00566.x.

Rubidge, E.M., and Taylor, E.B. 2005. An analysis of spatial and environmental factors influencing hybridization between native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*) in the upper Kootenay River drainage, British Columbia. Conserv. Genet. **6**(3): 369–384. doi:10.1007/s10592-005-4972-4.

Spies, I.B., Brasier, D.J., O'Reilly, P.T.L., Seamons, T.R., and Bentzen, P. 2005. Development and characterization of novel tetra-, tri-, and dinucleotide microsatellite markers in rainbow trout (*Oncorhynchus mykiss*). Mol. Ecol. Notes, **5**(2): 278–281. doi:10.1111/j.1471-8286.2005.00900.x.

Sprowles, A.E., Stephens, M.R., Clipperton, N.W., and May, B.P. 2006. Fishing for SNPs: a targeted locus approach for single nucleotide polymorphisms in rainbow trout. Trans. Am. Fish. Soc. **135**(6): 1698–1721. doi:10.1577/T05-291.1.

Stephens, M.R. 2007. Chapter 2: Comparative analysis of SNP and microsatellite estimates of rainbow trout introgression in native California golden trout. *In* Systematics, genetics and conservation of golden trout. Ph.D. dissertation, Graduate Group in Ecology, University of California, Davis, Calif.

Stephens, M.R., Clipperton, N.W., and May, B.P. 2009. Subspecies-informative SNP assays for evaluating introgression between native golden trout and introduced rainbow trout. Mol. Ecol. Resour. **9**(1): 339–343. doi:10.1111/j.1755-0998.2008.02407.x. PMID:21564644.

Templeton, A.R. 1986. Coadaptation and outbreeding depression. *In* Conservation biology: the science of scarcity and diversity. *Edited by* M.E. Soulé. Sinauer Associates, Sunderland, Mass. pp. 105–116.

Trotter, P. 2008 Cutthroat: native trout of the West. 2nd ed. University of California Press, Berkeley, Calif.

US Fish and Wildlife Service. 1975. Threatened status for three species of trout. Fed. Regist. **40**: 29863–29864.

US Fish and Wildlife Service. 2004. Revised recovery plan for the Paiute cutthroat trout (*Oncorhynchus clarki seleniris*). US Fish and Wildlife Service, Portland, Ore.

Wang, J. 2001. A pseudo-likelihood method for estimating effective population size from temporally spaced samples. Genet. Res. **78**(3): 243–257. doi:10.1017/S0016672301005286. PMID:11865714.

Wang, J. 2003. Maximum-likelihood estimation of admixture proportions from genetic data. Genetics, **164**(2): 747–765. PMID:12807794.

Wang, J. 2006. A coalescent-based estimator of admixture from DNA sequences. Genetics, **173**(3): 1679–1692. doi:10.1534/genetics.105.054130. PMID:16624918.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**(6): 1358–1370. doi:10.2307/2408641.

Williamson, K.S., Cordes, J.F., and May, B. 2002. Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Mol. Ecol. Notes, **2**(1): 17–19. doi:10.1046/j.1471-8286.2002.00129.x.

Wong, D.M. 1975. Aspects of the life history of the Paiute cutthroat trout, *Salmo clarki seleniris* Snyder, in North Fork Cottonwood Creek, Mono County, California, with notes on behavior in a stream aquarium. M.S. thesis, California State University, Long Beach, Calif.

Appendix A

Figure A1 shows how our method is derived from the model of Chikhi et al. (2001). The conceptual model underlying their method (Fig. A1*a*) includes an admixed population h that is formed in a single admixture event. The allele frequencies at a locus in h at the time of admixture are denoted by p_h^a (the superscript a denotes "at the time of admixture"), which is a weighted average, $\gamma p_1^a + (1-\gamma)p_2^a$, of the allele frequencies in populations 1 and 2 at the time of admixture. γ is the admixture proportion from population 1. Subsequent to the admixture event, T generations elapse before genetic samples are taken from the three populations. At the time of sampling, the allele frequencies in the three populations are no longer p_h^a, p_1^a, and p_2^a; rather, each population has experienced genetic drift to an extent determined by the scaled time $t = \frac{T}{2N_e}$, resulting in allele frequencies p_h^s, p_1^s, and p_2^s (the superscript s denotes, "at the time of sampling"). This model can be represented more formally as a directed acyclic graph (DAG) (Fig. A1*b*), in which each unobserved variable in the model is represented by an unshaded node and each observed variable by a shaded node (for an overview of the use of DAGs to represent joint probability models, see Jordan 2004).

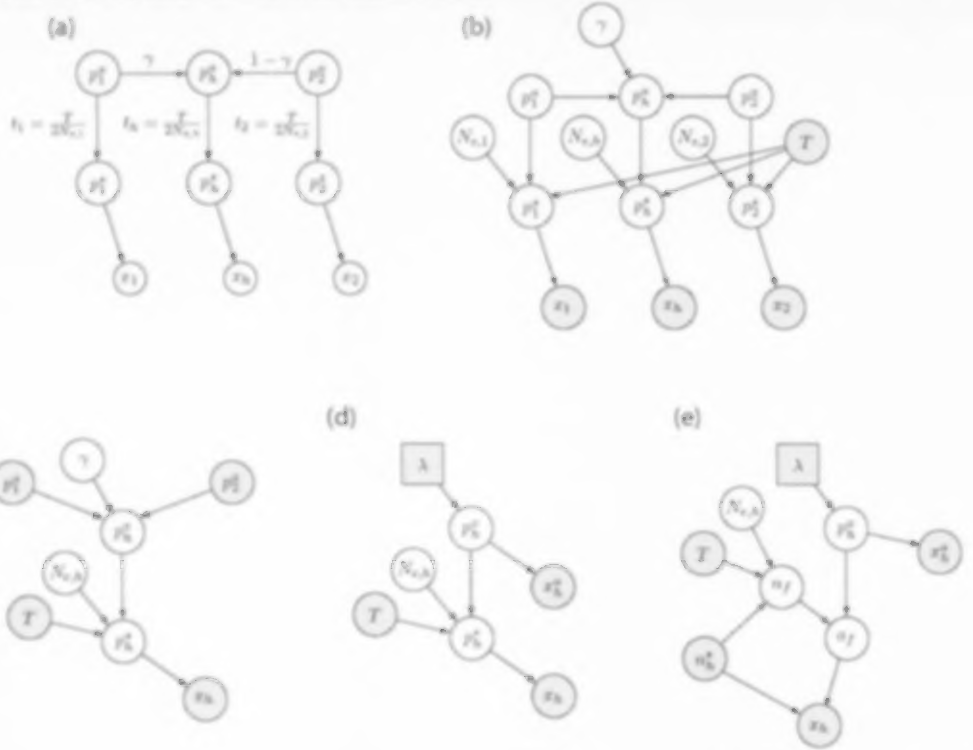
When using species-diagnostic SNP markers, the source populations are assumed fixed for alternate alleles, and hence the genetic drift in those populations is inconsequential. Accordingly, the allele frequencies in those populations at the time of admixture can be taken as observed quantities, simplifying the problem to the DAG shown in Fig. A1*c*. As before, p_h^a is exactly $\gamma p_1^a + (1-\gamma)p_2^a$. It is convenient to express this equality in terms of a probability density for p_h^a, which has all of its mass on the point $\gamma p_1^a + (1-\gamma)p_2^a$. Such a density is precisely the posterior density that would be obtained for p_h^a if an infinitely large sample were taken from the admixed population at the time of admixture and found to contain alleles in the proportions of $\gamma p_1^a + (1-\gamma)p_2^a$. We can therefore represent the model in Fig. A1*c* as the model in Fig. A1*d*, in which is endowed with a prior distribution that is a beta density parameterized by λ (we use $\lambda = \left(\frac{1}{2}, \frac{1}{2}\right)$ — the unit-information beta prior), and x_h^a is defined to be a very large, imaginary sample of size m gene copies taken from the admixed population at the time of admixture with proportions of alleles observed given by $\gamma p_1^a + (1-\gamma)p_2^a$. As $m \rightarrow \infty$, the models in Fig. A1*d* and Fig. A1*c* become identical and, in practice, are indistinguishable for large values of m such as $m = 10\,000$.

The final step in our derivation is to consider the drift in the admixed population from the perspective of the coalescent. The model shown in Fig. A1*d* considers drift in the classical, forward-in-time sense: the allele frequency in the population drifts during the time between the admixture event and the event of sampling . A different way of incorporating the effect of genetic drift in that time interval is to consider its effect on the unobserved coalescent tree connecting the gene copies sampled in x_h, as was introduced by Berthier et al. (2002). Doing so leads to the DAG in Fig. A1*e*. In words, allelic counts x_h from n_h^s gene copies sampled from the admixed population are observed at the present time. These n_h^s gene copies descended from n_f gene copies present at the time of admixture. n_f is unobserved, but its distribution is governed by the coalescent process running back in time for T generations with coalescent rate determined by $N_{e,h}$. The distribution of allelic types in the present-day sample is determined by a_f — the allelic types of the n_f ancestral gene copies (it is assumed there is no mutation occurring within the T generations) — and the properties of the coalescent process. Finally, the allele frequencies at the time of admixture are considered unobserved, but are modeled as if we had a sample x_h^a of size m (with m large) gene copies taken at the time of admixture and we observe alleles in that sample in the proportions of $\gamma p_1^a + (1-\gamma)p_2^a$. It is important to note that x_h^a is a function of γ.

The above model is identical in structure to that in Anderson (2005) in which the likelihood for N_e is computed given two temporally spaced samples — an "historical sample" and a "present-day sample." Complete mathematical details of the Monte Carlo approximation of the likelihood can be found in that paper. We compute the likelihood using the program CoNe described by Anderson (2005). In practice with L diagnostic SNPs, to find the joint likelihood for any pair of values $(\gamma^*, N_{e,h}^*)$, we set $m = 10\,000$ and then create an input data set for CoNe in which the historical sample is of size m gene copies in allelic proportions $\gamma p_1^a + (1-\gamma)p_2^a$ and the present-day sample is just our sample x_h. For diallelic loci, the calculations done by CoNe are exact, so only a single Monte Carlo replicate needs to be done. With repeated calls to CoNe the likelihood over a fine grid of points in the space of γ and $N_{e,h}$ can be calculated in a few seconds, efficiently delivering the joint likelihood for γ and $N_{e,h}$. We construct a confidence interval for the joint estimate of γ and $N_{e,h}$ from this likelihood surface by using the contour that is three units below the likelihood at the maximum likelihood estimate. This approximately corresponds to a 95% confidence interval.

In the supplemental material[1], we present a series of simulations showing that our method gives good maximum likelihood estimates of γ and $N_{e,h}$ and that the confidence intervals on $N_{e,h}$ behave well. The supplement also considers the application of this admixture model to scenarios where there may have been recurrent admixture over time rather than a single admixture event, but great uncertainty about the exact history of admixture. In connection with this, it is worth noting that the degree of drift increases linearly with T (the number of generations assumed since admixture) and inversely with N_e. As a consequence, estimates of N_e made using one value of T can be directly scaled to another value of T (e.g., if one assumes T to be two times longer, than the estimate of N_e will be two times larger).

Fig. A1. Graphical depiction of the derivation of the likelihood model used in this paper. (*a*) The conceptual model of Chikhi et al. (2001). (*b*–*e*) Directed acyclic graphs depicting derivation of our likelihood model.



References

Anderson, E.C. 2005. An efficient Monte Carlo method for estimating N_e from temporally spaced samples using a coalescent-based likelihood. Genetics, **170**(2): 955–967. doi:10.1534/genetics.104.038349. PMID:15834143.

Berthier, P., Beaumont, M.A., Cornuet, J.M., and Luikart, G. 2002. Likelihood-based estimation of the effective population size using temporal changes in allele frequencies: a genealogical approach. Genetics, **160**(2): 741–751. PMID:11861575.

Chikhi, L., Bruford, M.W., and Beaumont, M.A. 2001. Estimation of admixture proportions: a likelihood-based approach using Markov chain Monte Carlo. Genetics, **158**(3): 1347–1362. PMID:11454781.

Jordan, M.I. 2004. Graphical models. Stat. Sci. **19**(1): 140–155. doi:10.1214/088342304000000026.

Bayesian integrated survey-based assessments: an example applied to North Sea herring (*Clupea harengus*) survey data

Richard Hillary

Abstract: Stock assessments that use only fisheries independent data have been developed from a number of different standpoints over the last 15 years. While the ability of such stock assessments to avoid the use of potentially compromised or hard to interpret commercial data or making certain assumptions that are common to traditional assessments has been established, little has been made of their potential for detecting and estimating complex mortality trends over time or their potential utility in survey-based management procedures. Using North Sea herring (*Clupea harengus*) data as an example, a Bayesian survey-based assessment method that is able to estimate all the key population variables is detailed. However, survival probability, and not fishing mortality that is conditional on natural mortality, is the key parameter. Reversible jump Markov chain Monte Carlo routines were developed to explore the range of ages over which survival separates into year and age effects (a common assumption in many stock assessments). Post hoc estimates of natural mortality suggest that changes over years and ages may have occurred in relativity to historic levels. The derivation of reference points based on survival probability and surplus biomass production are detailed as proxies for more common *F*-based reference points. The potential role for the outputs of such assessments in a management procedure sense is discussed.

Résumé : Au cours des 15 dernières années, on a mis au point des évaluations de stocks qui emploient seulement des données indépendantes de la pêche et qui présentent un nombre de perspectives différentes. Leur capacité à éviter d'utiliser des données commerciales compromises ou difficiles à interpréter et à éviter de faire certaines présuppositions courantes dans les évaluations traditionnelles est bien établie; on s'est cependant peu intéressé à leur potentiel pour détecter et estimer des tendances complexes de mortalité en fonction du temps et à leur utilisation potentielle dans les procédures de gestion basées sur les inventaires. En utilisant des données sur les harengs (*Clupea harengus*) de la mer du Nord comme exemple, nous présentons les détails d'une méthode bayésienne d'évaluation basée sur les inventaires qui est capable d'estimer toutes les variables démographiques importantes, mais dans laquelle la probabilité de survie, non la mortalité due à la pêche conditionnelle à la mortalité naturelle, est la variable principale. Nous avons mis au point des routines de Monte Carlo par chaîne de Markov avec saut réversible pour explorer la gamme des âges durant lesquels on peut séparer les effets de l'année et de l'âge sur la survie (une présupposition commune dans plusieurs évaluations de stocks); les estimations post-hoc de la mortalité naturelle indiquent que des changements ont pu se produire au cours des années et des âges par rapport aux niveaux historiques. Nous présentons la dérivation de points de référence basés sur la probabilité de survie et la production de biomasse excédentaire comme variables de remplacement des points de référence plus courants basés sur *F* et nous discutons du rôle potentiel des résultats de telles évaluations dans une perspective des procédures de gestion.

[Traduit par la Rédaction]

Introduction

The concept of integrated stock assessment (Hilborn and Walters 1992; Quinn and Deriso 1999; Hampton and Fournier 2001) is that the available data be brought together in some sort of estimation framework (preferably statistical) and that the key parameters of the population and fishery model be estimated simultaneously: conditionally on the data and assumptions. In a general sense this is entirely sensible, but there are specific cases where one might not want to do this; for example, if subsets of the available data that must be used when one is to estimate certain parameters are flawed in some manner: more specifically if they are incomplete or biased. Obvious examples would be the use of biased catch data (e.g., lacking discard information) when trying to estimate fishing mortality and exploitation rates and if the assumptions required to estimate certain parameters or processes are invalid. Examples of this would be the assumption of a value of natural mortality without the data to estimate it or the assumption of fixed growth (mass-at-length / length-at-age) over time.

Over recent years, the work done in Cook (1997), Porch et al. (2006), Irwin et al. (2008), and most recently, Bogaards et al. (2009), developed methodologies for using fishery independent data and relative abundance population models to es-

Received 10 June 2010. Accepted 18 March 2011. Published at www.nrcresearchpress.com/cjfas on 12 August 2011.
J21870

Paper handled by Associate Editor Ray Hilborn.

R. Hillary.* Fisheries Group, Division of Biology, Imperial College London, Ascot SL5 7PY, UK.

E-mail for correspondence: Rich.Hillary@csiro.au.

*Present address: CSIRO Marine and Atmospheric Research, Hobart 7001, Tasmania, Australia.

Can. J. Fish. Aquat. Sci. **68**: 1387–1407 (2011) doi:10.1139/F2011-047 **Published by NRC Research Press**

timate biomass and recruitment trends and fishing mortality values (conditional on assumed natural mortalities) in the absence of catch data. The work of Needle (2005) and Beare et al. (2005) goes further by not assuming a value of natural mortality at all and simply estimating recruitment, biomass, and total mortality-at-age. In Trenkel (2008), an even simpler model was assumed that uses juvenile and adult survey data to separate the dynamics of the adult biomass into recruitment and growth or decline random effects.

In the models suggested by Bogaards et al. (2009), Cook (1997), Porch et al. (2006), and Needle (2005), a separable model was assumed for the total mortality rate or for the fishing mortality if an assumed level of natural mortality, M, was used to separate fishing mortality, F, from total mortality, $Z = M + F$. When the vector of natural mortality-at-age is to be assumed as known (Cook 1997; Porch et al. 2006; Bogaards et al. 2009), it is not permitted to change from year to year; when it is not assumed to be known and a separable total mortality-at-age is estimated (Beare et al. 2005; Needle 2005), this imposes strict conditions on how both natural and fishing mortality-at-age are permitted to vary from year to year. In this paper, survival probabilities due to natural causes and exploitation are not separated, and a flexible nonseparable model is permitted. There are many reasons why a separable model for fishing mortality may not be applicable, such as changes in fleet structure and fishing pattern and changes in the spatial distribution of the fish population-at-age, but there are just as many, if not more, reasons for survival rates due to natural causes to vary, not just by age or length but by year also. For a species that, at least over some ranges of ages, is a major prey item in a given ecosystem, the natural mortality rate may vary owing to changes in predator abundance, depending on whether the predation uptake function is density dependent. Also, changes in relevant environmental conditions could conceivably affect the population survival rates; changes in growth with a natural mortality rate related to length can easily result in a change in the natural mortality-at-age.

For obvious reasons, in more traditional stock assessment models that utilize catch, effort, survey, and other forms of data, it has been something of a necessity to assume that natural mortality (and often growth) does not vary with time. This does not mean that natural mortality cannot ever be estimated, but simply that it can be difficult to estimate in conjunction with other parameters of interest that strongly depend on or correlate with natural mortality. While the impact of random variations in mortality and growth may often be absorbed into the uncertainty in the estimated parameters, this does not mean that key reference points — MSY being a clear example — estimated from such models are not then prone to potentially large biases if this variation is nonrandom. Whether basing management action on or assessing stock status relative to such biased reference points, it is not difficult to imagine the problems that could arise. The ecosystem approach to resource management has become a key area of both public interest and research (Christensen et al. 2000; Fulton et al. 2005; Watters et al. 2005) in recent times. The wish to manage an exploited resource, as well as the impact of the exploitation on related ecosystem components, has been advocated at a global level (i.e., United Nations Earth Summit) and aspects of this approach are already being operationalized in certain fora, such as the Commission for the Conservation of Antarctic Marine Living Resources. What is less common is to account more explicitly for the affect of the surrounding ecosystem on the resource being exploited when performing an assessment of a given stock. Either simplifying assumptions must be made to reduce our particular model to something that is conceivably estimable from the available data or one looks to develop complex multitrophic coupled models to describe the entire ecosystem where many parameters are required and most are difficult if not impossible to estimate.

While there are obvious potential benefits to be gained by using survey data alone to assess the status of a given population (Cotter 2009; Mesnil et al. 2009), I propose that they also permit us to explore the impact of assumptions that so often have to be made in more traditional stock assessment methods. In particular, one need not be constrained by assumptions about how mortality-at-age, both fishing and natural, varies with time and in essence permits a blind approach to single-species, ecosystem-cognizant assessment: changes in mortality (and vital) rates can be detected, but their drivers can still remain unknown. Using the wide array of survey data (egg, acoustic, and trawl) available for North Sea herring, a flexible assessment method is demonstrated that uses Bayesian and Markov chain Monte Carlo (MCMC) methods to estimate the magnitude of and probabilistic evidence for annual and age-specific variance in herring survival rates. I compare the results of the proposed method with those coming from the current tuned virtual population analysis (VPA) assessment used on the example stock. The evidence for dynamics not currently permitted in the current herring assessment is also detailed.

Materials and methods

Data

For North Sea herring, there are three key survey data sets that are used in the analyses that follow.

Acoustic survey

Raw data are in terms of numbers-at-age (ages 1–9 and from 1989 to 2006), but in the probability model the proportions-at-age and the biomass time series are used. Detailed information on the survey design can be found in International Council for the Exploration of the Sea (2007). The survey is conducted annually from late June to early August during daylight hours (to account for herring diurnal migration), using standardized and calibrated equipment across vessels, each with constant speed, and with transects no more than 15 nautical miles (1 nautical mile = 1.852 km) apart. The survey was designed to cover the whole spatial range of the stock.

International Bottom Trawl Survey (IBTS)

Raw data are in terms of survey catch-per-unit-effort (CPUE)-at-age (ages 1–5 and from 1984 to 2006), but, as with the acoustic data, the proportions-at-age and the biomass time series are used in the estimation scheme. These data are collected as part of a multinational groundfish trawl survey of the whole of the North Sea, primarily directed at younger and more shallow-dwelling fish. Regarding herring, the older and fully mature are covered very poorly, if at all.

Multiplicative Larval Abundance Index (MLAI) survey

Estimates of the relative abundance of eggs were assumed to be a proxy for the spawning stock biomass. Several 2-week-long surveys (all with the same operational design and relative to known spawning times and areas) where larval samples of herring (<17 mm) are binned and a daily hatch rate calculated per 1 mm bin were performed. Multivariate analysis of variance (MANOVA) was then used to compensate for missing data in the spatiotemporal strata and a standardized relative larval abundance per year is derived (Patterson and Beveridge 1995).

Population dynamics model

For reasons that will be clarified later, a preferred alternative would be to model the probability of survival, $\pi^s_{y,a}$, and not instantaneous mortality, as follows:

$$(1)\qquad N_{y+a-r,a} = R_y \prod_{i=r}^{a-1} \pi^s_{y+i-r,i}$$

In this particular example, a plus group is not assumed and the maximum age class is the terminal age, although a plus group would be simple to account for in the usual manner. For the initial nonrecruit numbers-at-age, the following model is assumed:

$$(2)\qquad N_{y_{\min},a} = N_{y_{\min},a-1}\pi^s_{y_{\min},a-1}\exp\left(\varepsilon^n_a - \sigma^2_n/2\right)$$

where σ_n is a standard deviation penalising the deviation from equilibrium in the initial numbers-at-age vector. For the case where absolute abundance is to be estimated, the following model for recruitment is envisaged:

$$(3)\qquad R_y = \overline{R}\exp\left(\varepsilon^r_y - \sigma^2_r/2\right)$$

where $\varepsilon^r_y \sim N(0,\sigma^2_r)$. Here, $\overline{R}$ is the mean absolute recruitment level, and for the case where relative abundance is estimated, one assumes that $\overline{R} \equiv 1$. The incorporation and reliable estimation of a stock–recruit relationship is a difficult problem in any stock assessment, perhaps more so in assessments such as this one:

If the survey data do not cover the historically exploited time scales, we must either make assumptions about the initial depletion status and survival probability levels of the stock at the start of the analysis relative to unfished levels, or try to estimate both current starting abundance and the unfished level based on the stock–recruitment signal. Without absolute abundance data we cannot estimate the full stock–recruit relationship. All that could realistically be achieved (if a signal is present in the data) is to estimate the depletion ratio (relative to an arbitrary unfished level) and the per-unit-spawning potential reduction in recruitment (via steepness for example). For the herring case detailed in this work, the first point is an issue (analysis begins in 1989) and, as we shall see later, there is no observable informative relationship between spawning stock biomass (SSB) and recruitment. Simple inspection of the survey data shows, very clearly, that the system was not at some kind of equilibrium at the start of the data coverage, meaning we would, in the Beverton–Holt paradigm, have to estimate virgin SSB, steepness, and initial abundance. Even with a reasonably informative prior for steepness, we are still left with a problem: without some kind of signal in the data on recruitment changing in direct relation to SSB levels there will be no way to sensibly estimate these two abundance parameters. It is for these reasons why I do not assume a stock–recruit relationship for the particular example stock and data set. Furthermore, the particular assessment model in this paper is not suitable for any kind of future scenario modelling, as the impact on recruitment of abundance levels outside the range of those observed herein cannot be characterized without further assumptions.

A flexible model for the survival probabilities is permitted within the model whereby I separate the years and ages into separable and nonseparable groups. For a given set of years and ages, Ω, covered by the data $\Omega = Y \times A$, where Y is the set of years and A is the set of ages for the ages $A_S \subseteq A$ and years $Y_S \subseteq Y$, which are to be considered separable, the following model is assumed:

$$(4)\qquad \operatorname{logit}(\pi^s_{y,a}) = \log\left(\frac{\pi^s_{y,a}}{1-\pi^s_{y,a}}\right) = \gamma^s_{y,a} = \eta_y + \chi_a$$

where η_y and χ_a are the year and age effects, respectively. This is essentially the same assumption one would make for a logistic regression model, making it ideally suited to modelling probabilities, but the other reasons for assuming such a model as the one described in eq. 4 are as follows. (*i*) In terms of MCMC performance estimating these parameters instead of two probabilities (i.e., constrained between 0 and 1) multiplied together makes more sense. They are less correlated and the particular random walk MCMC sampler is more efficient on an unbounded domain. (*ii*) For a separable fishing mortality model it is common to estimate a constrained functional form, thus reducing parameters and incorporating gear-specific knowledge. In this particular context this parameter will be a combination of age effects owing to natural and fishing mortality; the former normally tends to decrease with age (length) while the latter sometimes tends to increase (lower availability – targeting of younger – shorter fish). Given this, it seems sensible to permit a reasonably free model for the age effects.

It is worth being clear on how the separable model for survival probability assumed here is different from the separable model for total mortality assumed in previous work (Beare et al. 2005; Needle 2005), where it is assumed that $Z_{y,a} = z_y\kappa_a$. If $Z_{y,a} = m_y\kappa^m_a + f_y\kappa^f_a$, then there are only three ways that such a form can be considered separable in terms of being able to model the process as $Z_{y,a} = z_y\kappa_a$: (*i*) if the age effects due to natural and exploitation mortality are different, then m_y and f_y **must** change by exactly the same proportion each year or relative to each other in a uniquely defined manner so as to maintain a constant proportion-at-age in the total mortality vector, (*ii*) either m_y or f_y is equal to zero, or (*iii*) the age effects due to natural mortality are exactly the same as their exploitation-related counterparts, or there are no age effects at all. Only option *iii* sounds plausible and even then in presumably very few restricted cases. The same problem would appear with the following survival model: $\pi^s_{y,a} = \phi_y\kappa_a$, as this assumption also imposes a fixed proportion-at-age in the survival probability across years. The separable model defined in eq. 4, where survival-at-age separates into year and age effects modelled in logit space does not induce the same condition on the survival rate-at-age by year: the proportions-at-age surviving need not be constant across years. If one were to decompose the two sources of mortality as follows:

$$(5)\qquad \gamma^{s}_{y,a} = \eta^{m}_{y} + \chi^{m}_{a} + \eta^{f}_{y} + \chi^{f}_{a}$$

where the superscripts *m* and *f* refer to survival effects due to natural and fishing-related causes, respectively, then if one could not separate the two sources of mortality (e.g., we did not know the natural mortality effects) one would collapse back to the model in eq. 4. As an aside, it also makes the inclusion of covariates related to both natural (environmental drivers, predator abundance) and exploitation (effort, capacity) mortality effects in eq. 5 quite simple. The main point being that the age effects of the two sources are perfectly free to be different, as are the year effects, whilst still being consistent with the underlying survival model. The key factor is that the model for total mortality normally assumed is multiplicative in terms of the year and age effects but in terms of the natural and fishing effects it is additive. One note of caution with regards to interpretation: in logit-space the survival effects are linear and a change in age–year effect will have no effect on the year–age specific parameters $\gamma^{s}_{y,a}$ but in real space a change in the year effect will have an age-specific impact on survival probabilities in that year, as will age effect changes relative to the overall year-specific survival rate.

The final issue relating to the survival probabilities is how one constructs the relevant values in the terminal years and ages. For all the ages in the final year and for all the years at the final age we cannot estimate the survival probabilities as the information on these parameters comes primarily from the relative change in the proportions-at-age from one age class to the next the following year as noted in Cook (1997) and Needle (2005). These parameters can be required to compute model-predicted quantities, such as SSB and survey biomass indices and proportions-at-age, if these surveys do not occur at the time at which we define a year to begin. If I permit a flexible separable and nonseparable survival model, I must deal with these two cases separately. For the separable case the final year effect η_Y is defined to be a 3-year back average of the previous year effects; the final age effect is defined to be the same as the penultimate age effect: $\chi_A = \chi_{A-1}$. In the nonseparable case for the terminal years again a 3-year back average is employed to define $\pi^{s}_{Y,a}$, and for the terminal ages a simpler model is assumed so that $\pi^{s}_{y,A} = \pi^{s}_{y,A-1}$. There was no definitive biological or statistical reason to choose 3 years as the averaging period, merely that an averaging period makes more sense for the terminal years than it does for the terminal ages, where there is little to no growth and where few animals reach. For either the separable or nonseparable models these assumptions are effectively identical. In the terminal years one need only average the year effect; for the terminal age only the age effect.

SSB (relative and absolute) is calculated as follows:

$$(6)\qquad \mathrm{SSB}_{y} = \sum_{a} m_{y,a} w_{y,a} N_{y,a} (\pi^{s}_{y,a})^{\tau_{sp}}$$

where τ_{sp} is the time (as a fraction of a year) at which spawning occurs, and $m_{y,a}$ and $w_{y,a}$ are the proportions mature and masses-at-age, respectively.

Probability models for the data and parameters

As mentioned the age-structured data are separated into two components: proportions-at-age and total biomass. The MLAI survey data, as in the current assessment (International Council for the Exploration of the Sea 2008), are assumed to be proportional to the model-predicted SSB. For the survey age proportions one can do a preassessment of the likely information content, with respect to mortality and survival probability, by looking at a scatterplot of the log-transformed index by year and age. Figures 1*a* and 1*b* show these summaries for the acoustic and IBTS data, respectively. When looking at the acoustic data one sees a fairly coherent pattern in that the scatterplot of each age class in (across years) shows a strong linear trend which is indicative of a strong mortality signal in the data. For the IBTS data, there is a similar general linear trend but it is noisier, driven by the lesser precision of the IBTS data relative to the acoustic data — especially at the older ages. This does not give us the relevant information from which we can derive immediate estimates of total mortality or survival, and we have not yet dealt with the issue of the vulnerability of the population to the survey gear, but at least confirms that such information should in principle be contained within the data. With respect to the biomass trend in each of the three surveys, we see a general agreement in trend (in particular the biomass decline and recovery in the 1990s) but some differences in trend in more recent years (see Fig. 2). This completes the exploratory data analysis and now I deal with the probability models, where a state–space structure is assumed, for the various data sources.

Dealing with the age composition data first, for a given survey one has observed proportions-at-age (or length–stage as the treatment is exactly the same) $p_{y,a}$ and the index-at-age, $I_{y,a}$, can be represented as a multinomial variable:

$$(7)\qquad I_{y,a} = E^{m}_{y} p_{y,a}$$

The variable E^{m}_{y} is called the effective sample size — normally smaller than the true number of sampled fish — which represents the number of fish sampled randomly within the survey. For each of the surveys there are observation–sampling error covariance matrices-at-age available, and from these one can obtain the coefficient of variation-at-age, CV_a, and given the assumption that the survey data are multinomial, the following relationship can be used:

$$(8)\qquad \mathrm{CV}_{a} = \frac{\sqrt{E^{m}_{y} p_{y,a}(1-p_{y,a})}}{E^{m}_{y} p_{y,a}} = \sqrt{\frac{1-p_{y,a}}{E^{m}_{y} p_{y,a}}}$$

One can obtain an estimate of the value of N_y by minimizing the sum of squares (summed across ages) of the individual values in eq. 8 as done in Hillary et al. (2006). From the survey data, stratified subsampling at strata below the year and age level yield an observation error covariance matrix and associated CV-at-age for the survey data. From eq. 8 we see that a multinomial distribution has a specific CV-at-age equation, conditional on the proportions (which we know) and the effective sample sizes (which we do not know). Using

Fig. 1. Scatterplot by age (across years) for (*a*) the acoustic survey and (*b*) the International Bottom Trawl Survey (IBTS). For each panel (denoting age), the plot is simply the annual raw survey data at that age, conditional on the data at all the other ages.

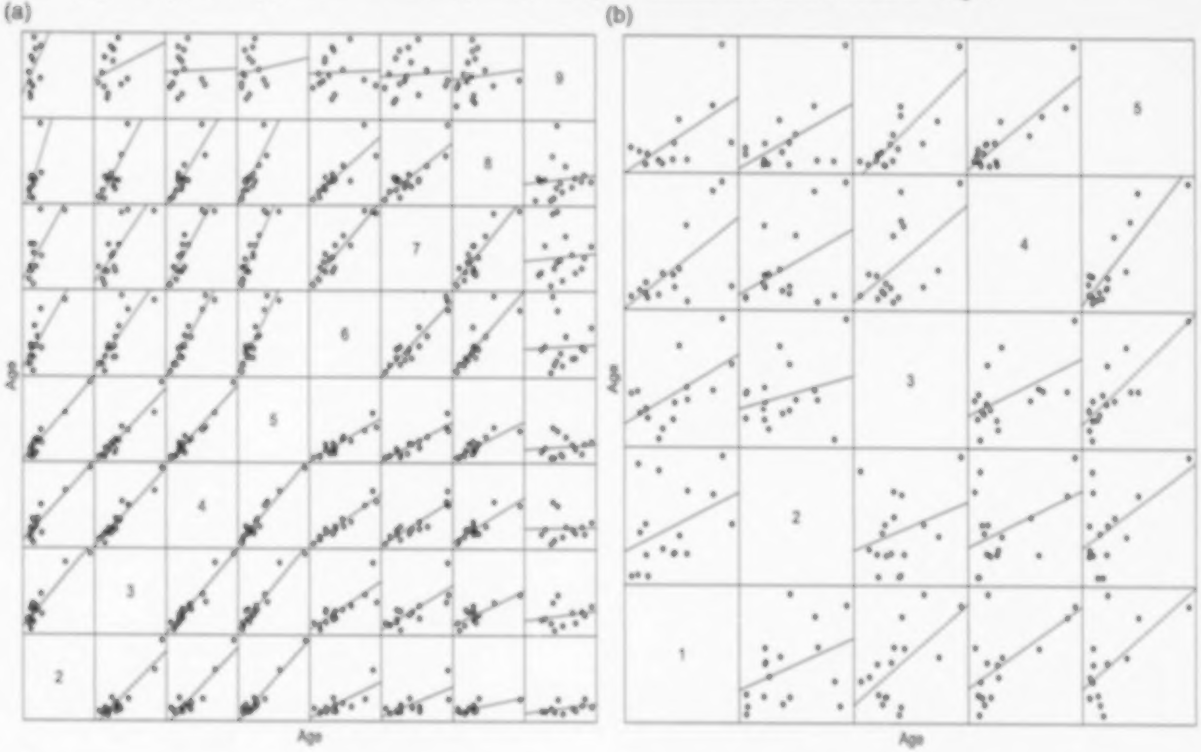
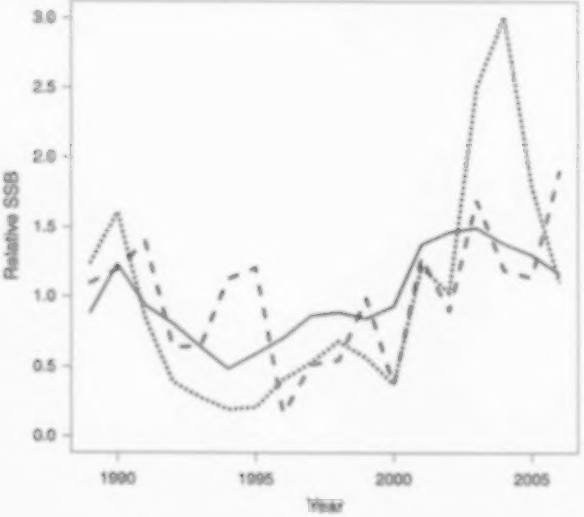


Fig. 2. Relative (mean-standardized) trends in mature biomass for each of the three surveys used in the assessment: acoustic (solid line), Multiplicative Larval Abundance Index (MLAI, dashed line), International Bottom Trawl Survey (IBTS, dotted line).



the CV-at-age estimates from the survey, one can then simply solve for the effective sample sizes that best represent the observed CVs, given the assumption of a multinomial probability model. The CVs-at-age (Table 1) and the associated estimates of effective sample size for the acoustic and IBTS data (Table 2) are detailed. For a given survey the model-predicted index-at-age is derived as follows:

$$(9)\qquad \widehat{I}_{y,a} = q\zeta_a N_{y,a}(\pi^t_{y,a})^{\tau_{surv}}$$

where q is a constant of proportionality, ζ_a is the vulnerability of the population to the survey gear, and τ_{surv} is the time of year the survey is undertaken. From eq. 9 it is simple to derive model-predicted proportions-at-age, and the basic multinomial likelihood for the survey age data can be expressed as follows (with the •,• notation relating to coverage over both years and ages):

$$(10)\qquad L^{age}(I_{\bullet,\bullet}|\cdots) = \prod_y \frac{E^{ss}_y!}{\prod_i E^{ss}_y p_{y,i}!}\prod_j \tilde{p}_{y,j}^{E^{ss}_y p_{y,a}}$$

This is, however, a reasonably rigid assumption, and in particular the model-predicted variance in these data will be constrained by the sample size and the predicted proportions-at-age only as $\tilde{p} \equiv \widehat{p}$, the model-predicted proportions-at-age. This does not permit the probability model to account for variance in the data different to the level one expects from a multinomial distribution. To be able to account for such a process, one can assume the multinomial proportions-at-age to be random variables themselves following a Dirichlet distribution (and, henceforth, $\Gamma(\ldots)$ refers to the gamma function):

Table 1. Observation error CV-at-age for the International Bottom Trawl Survey (IBTS) and acoustic survey data.

	Age								
	1	2	3	4	5	6	7	8	9
Acoustic	NA	0.188	0.192	0.217	0.223	0.233	0.245	0.256	0.301
IBTS	0.216	0.282	1.65	2.058	1.761	NA	NA	NA	NA

Note: These CVs were then used to derive the multinomial effective sample sizes in Table 2.

Table 2. Multinomial effective sample sizes for the IBTS and acoustic age data given the survey CV-at-age in Table 1 and the equation detailed in eq. 9.

Year	Acoustic	IBTS
1989	456	18
1990	322	8
1991	264	8
1992	228	10
1993	202	15
1994	217	14
1995	311	21
1996	357	32
1997	406	27
1998	339	24
1999	362	9
2000	260	26
2001	458	13
2002	334	24
2003	437	11
2004	296	8
2005	305	7
2006	282	7

$$P(\widetilde{p}|\omega,\widehat{p}) = \frac{\Gamma\left(\sum_i \omega\widehat{p}_i\right)}{\prod_i \Gamma(\omega\widehat{p}_i)} \prod_i \widetilde{p}_i^{\,\omega\widehat{p}_i - 1} \tag{11}$$

Multiplying eqs. 10 and 11 gives us the joint density of the survey data and the random proportions-at-age $\widetilde{p}$. To obtain the marginal likelihood of the survey data, one must first integrate over the random proportions and one finds that

$$L^{age}(I_{\bullet\bullet}|\cdots) = \Xi \prod_y \prod_i \frac{\Gamma(E_y^{ss} p_{y,i} + \omega\widehat{p}_{y,i})}{\Gamma(\omega\widehat{p}_{y,i})} \tag{12}$$

where Ξ is a constant of proportionality not involving any model-predicted variables. This may seem a complex treatment of the data but is a common way to extend the multinomial probability model (Polacheck et al. 2006), and the key advantages lie in the mean and variance model predictions for the data:

$$\begin{aligned} E(I_{y,a}) &= E_y^{ss}\widehat{p}_{y,a} \\ V(I_{y,a}) &= \varphi E_y^{ss}\widehat{p}_{y,a}(1-\widehat{p}_{y,a}) \end{aligned} \tag{13}$$

so one obtains the same mean value as that predicted by the multinomial distribution but a variance scaled by the dispersion parameter, φ:

$$\varphi = \frac{E_y^{ss}(\omega + 1)}{E_y^{ss} + \omega} \tag{14}$$

This affords one the freedom to alter the parameter ω (which can be interpreted as the magnitude of the extra variation in the proportions) to make sure the model-predicted variance matches that seen in the real data — the process of calculating the correct value of ω will be dealt with later.

The acoustic and IBTS biomass indices are not derived via a model-based standardization routine as CPUE biomass indices traditionally are; they are obtained from the age-specific survey data as follows:

$$I_y = \sum_a I_{y,a} w_{y,a} \tag{15}$$

and are assumed to be distributed around the model-predicted biomass indices according to a normal-log (as opposed to a log-normal) distribution, and the reasons for the slight change in the usual assumed likelihood for such data will be explained in the section detailing the MCMC algorithm used. For the MLAI survey, this index is assumed to follow a normal-log distribution about the model-predicted SSB. The variance of this distribution is assumed to be an additive combination of observation and process error:

$$\sigma_I^2 = \sigma_y^2 + \sigma_P^2 \tag{16}$$

where σ_y^2 is the sampling error (by year if available or calculable) and σ_P^2 is the process error variance — the extra variance beyond sampling error required by the model to account for the variance in the data. The yearly sampling error terms for the acoustic survey and IBTS data were derived by taking the available CV-at-age for each index and computing a weighted mean CV across the ages with weights equal to the proportions-at-age in the survey for a given year. For the MLAI survey, a single estimate of the survey sampling error CV (equal to 0.219) was available and was applied across all years.

One issue that must be addressed is the vulnerability of the population to the survey gears. For the acoustic index, it is assumed in this work that the whole population of the ages covered in the index can be captured by the survey gear. As is the case with other such works (Cook 1997; Needle 2005; Bogaards et al. 2009), it is not always feasible to estimate a vulnerability ogive per survey series, as they will clearly confound strongly with the age-related mortality–survival effects.

However, one can make certain assumptions about the vulnerability ogive in one survey and estimate the others relative to this assumption, although this is by no means guaranteed not to be confounded with the mortality age effects; this depends strongly on the information content in the survey where one assumes to know the vulnerability ogive. Given the fact that the IBTS series is directed at juvenile animals, it was assumed that the survey captures the first two age classes (ages 1 and 2) equally and fully and that the vulnerability of ages 3 and above decreases exponentially with age according to an estimable parameter, ψ, so $\zeta_a = \exp(-\psi a)$ for ages 3 and above.

Within models of this kind there are situations where one could estimate the vulnerability patterns, but it does depend on what form they take. For the case of surveys where at least one survey has a logistic-type vulnerability pattern, then it is plausible that one could estimate the relationships — the main problem comes with either monotonically decreasing or dome-shaped vulnerability patterns. The mortality signal is assumed to be a geometric–exponential decrease in a cohort's abundance over time, so if there is also some decrease in the survey's coverage as the ages increase, it will most likely be impossible to separate these two effects without an assumed natural mortality vector and catch–effort data (linked to exploitation rates). Without some sort of a priori information to tell us whether it is likely that the vulnerability patterns of any of the surveys will fit the identifiability criteria, one must either (*i*) assume or specify an informative prior for one of the survey vulnerability patterns (as done in this work) or (*ii*) use some other kind of abundance information (such as mark–recapture information as done in Irwin et al. 2008) with no such coverage issues and estimate the other vulnerability patterns relative to this information.

A Bayesian framework is employed in this work, meaning one must now define prior distributions for all the key estimable parameters. Dealing with the survival probability parameters, first recall a flexible model is permitted whereby certain years and ages can be considered as separable, whereas the remaining years and ages are free to vary from year to year and age to age. For the particular example, this freedom was restricted to only permitting the age classes to follow a separable model — if a set of age classes is considered separable, age effects are estimated for these age classes but with year effects estimated for every year. Clearly having only one separable age class is no different to having none (in terms of parameters estimated), so while the model covers ages 1 to 9, if any form of separable model is assumed, it must have a minimum of two age classes within it. Given the survival probability parameters represent the chance of occurrence of a binary process (survival or death), then in the absence of data, the following "uninformative" Jeffreys prior (Jeffreys 1961) is assumed:

$$(17) \qquad p(\pi^s) = (\pi^s)^{-1/2}(1-\pi^s)^{-1/2}$$

Henceforth, $p()$ denotes the prior distribution of the relevant parameters. As mentioned, the actual parameters estimated are the logit-transformed survival probabilities, γ^s, so one must use the change of variables technique to transform the prior in eq. 17 into a prior for the γ^s:

$$(18) \qquad p(\gamma^s) = p\left[\pi^s = \Phi^{-1}(\gamma^s)\right]\left|\frac{d\Phi^{-1}}{d\gamma^s}\right|$$

where Φ is the logit transformation. The formula in eq. 18 leads to the following density for γ^s:

$$(19) \qquad p(\gamma^s) = [1+\exp(\gamma^s)]^{-1/2}\left[\frac{\exp(-\gamma^s)}{1+\exp(\gamma^s)}\right]^{1/2} \times [\exp(\gamma^s)+\exp(-\gamma^s)+2]^{-1}$$

which reduces further to the following:

$$(20) \qquad p(\gamma^s) \propto \frac{1}{2}\operatorname{sech}(\gamma_s/2)[\exp(\gamma^s)+\exp(-\gamma^s)+2]^{-1}$$

where sech(…) is the hyperbolic secant function, and the density in eq. 20 details a symmetric prior density for γ^s on $(-\infty,\infty)$. For the separable case where γ^s decomposes into age and year effects, the joint prior density of η_y and χ_a is given by eq. 20, and assuming that $\gamma^s_{y,a} = \eta_y + \chi_a$, this imposes a negatively correlated joint prior on the separable parameters.

For the catchability coefficients (relating population to survey biomass), their natural logarithms are the estimated parameters with an assumed normal prior. In conjunction with the normal-log likelihood function, this gives us a conjugacy (Bernardo 2003) whereby the conditional posterior of the parameters is of a known form, in this case normal:

$$(21) \qquad P[\ln(q)|\cdots] = N\left\{\left[\frac{\mu_q}{\sigma_q^2}+\frac{\sum \ln(I_y/\hat{I}_y)}{\sigma_I^2}\right] \times \left(\frac{1}{\sigma_q^2}+\frac{\Theta_I}{\sigma_I^2}\right)^{-1}, \left(\frac{1}{\sigma_q^2}+\frac{\Theta_I}{\sigma_I^2}\right)^{-1}\right\}$$

where Θ_I is the number of years in the survey and μ_q and σ_q are the prior mean and standard deviation of ln(q), respectively, and P[] denotes the posterior distribution. Not only does this make sampling from the parameters' posterior much easier (as we shall see later), but it also makes the definition of informative–uninformative priors for q straightforward should they be required.

For the recruitment and initial equilibrium population deviations, a normal prior is assumed with mean zero and standard deviations $\sigma_r = 0.6$ and $\sigma_a = 0.25$, respectively. The value of the recruitment standard deviation was chosen as this value is often chosen for stocks that expect mild, but not overly strong, stochastic recruitment deviation; the value of 0.25 for the initial population structure standard deviation was chosen to permit the model enough freedom to explain the nonequilibrium age structure in the survey data. In the base-case model, the acoustic biomass index is assumed absolute, so the logarithm of the mean recruitment level, $\ln(\overline{R})$, is an estimable parameter and as such is assigned a uniform prior. The final parameter is the IBTS vulnerability ogive parameter ψ, and this parameter is assigned a uniform prior on the positive real numbers. For the process error standard deviations, σ_P, a log-uniform prior is assumed in all cases. The log-catchability priors are set at $\mu_q = 0$ and $\sigma_q = 10$ — very disperse and quasi-uninformative.

The final part of the objective function relates to the potential penalties employed — constraints on the system based upon subjective preference or in most cases common sense. The only penalty used is to ensure that the recruitment deviations from the first to the final year have mean zero across the years. This is done for a number of reasons primarily to avoid identification problems with the recruitment in the final year (little to no information on this in the data), but can also reduce the correlation of the mean recruitment level with the year-averaged mean recruitment deviates and ensure the actual recruitments average close to $\bar{R}$. The penalty is implemented by subtracting k times the square of the mean recruitment residuals (across years) from the log-posterior, and in this work a value of $k = 10$ was chosen. This, in conjunction with the base prior on each of the recruitment residuals, forms a correlated prior density for the residuals across years, and in the following the prior for these parameters will be implicitly assumed to denote the base prior and the penalty detailed here.

According to Bayes' theorem the posterior distribution of the parameter set, θ, is defined as follows:

$$P(\theta, X|D, K) \propto \left[\prod_{d \in D} L^d(I^d|\theta, X, K)\right] p(\theta) \tag{22}$$

where D is the set of data available with associated likelihood $L^d(\ldots)$, X are the process variables (derived from θ), K is the knowledge (the set of assumed known variables such as maturity, mass-at-age, and so on), and finally $p(\theta)$ denotes the full prior density. In most cases (including this one) the distribution in eq. 22 will not be of a known form, and although many techniques exist for making inference about θ given eq. 22, I use MCMC (Gilks et al. 1996) techniques to generate a sample from the parameter posterior of interest. One further complication is that I also allow for the separable nature of the model to change as well, which requires an augmented version of the posterior to take account of the existence of multiple models, m:

$$P(\theta^m, X^m, m|D, K) \propto \left[\prod_{d \in D} L^d(I^d|\theta^m, X^m, m, K)\right] \times p(\theta^m|m)\, p(m) \tag{23}$$

This formulation encompasses both parametric and structural–model uncertainty, as we are able to define a posterior distribution for the parameters and the relevant model together. Discriminating between candidate models in the Bayesian context has a number of proposed and applied methods: the deviance information criterion is a common choice being an analogue of sorts of the frequentist Akaike information criterion or Bayesian information criterion approaches (Spiegelhalter et al. 2002), but another alternative is the use of Bayes' factors (Kass and Raftery 1995), where the Bayes' factor for a particular model m_1 over another m_2 is defined as the (model) posterior odds divided by the (model) prior odds:

$$B(m_1, m_2) = \frac{P(m_1|\cdots)\, p(m_2)}{P(m_2|\cdots)\, p(m_1)} \tag{24}$$

The expression in eq. 24 can also be viewed as the ratio of the marginal likelihood of the data conditional on the relevant models and is obtained by integrating eq. 23 over the relevant parameter vectors, dividing by the model prior (purely data-driven), and then taking the ratio to derive the Bayes' factor. The Bayes' factor is useful as it gives us a possible way of not only deciding which model is "better" than another but the probability of that model, relative to another. It mimics the likelihood ratio but is averaged over the parameter distribution, not calculated at the maximum likelihood estimate. In terms of interpretation, a value of $B(m_1, m_2) > 1$ signifies more support in the data for model m_1 versus m_2, but in terms of absolutes, Jeffreys (1961) suggested that a Bayes' factor of between 1 and 3 was barely worth mentioning, between 3 and 10 as substantial, between 10 and 30 as strong, between 30 and 100 as very strong, and greater than 100 as decisive in terms of evidence for one model relative to another. One can also use the definition in eq. 24 to look at the relative evidence for one subset of models versus another. Let M_1 be a subset of models for comparison with another (not necessarily disjoint) subset of models M_2, then the Bayes' factor for each model subset can be thus defined:

$$B(M_1, M_2) = \frac{\int_{m \in M_1} P(m|\cdots) p^{-1}(m)\, dm}{\int_{m \in M_2} P(m|\cdots) p^{-1}(m)\, dm} \tag{25}$$

assuming that each model has nonzero support in the prior so that $p(m) > 0$ for all models m.

I used a reversible jump MCMC to allow estimation of the Bayes factors (Green 1995; see Appendix A for details) by sampling from the joint posterior of the model and the model-specific parameters. A two-step approach is detailed in the Appendix that shows how to (*i*) sample the parameters of a given model and (*ii*) define how to jump between different models. In terms of the models permitted, only the number of ages to be considered separable was considered as a parameter (thus defining the model), not the years as well, so if a set of ages was considered separable then they were assumed to be so for all years. While it is possible that the more complex situation where a set of both years and ages were considered separable, this would lead to over a hundred candidate models and, as such, was not attempted. In terms of prior distributions for each of the models, given M possible models the prior density of any particular model, m, is equal to $1/M$ so all models were a priori equally probable.

Posterior predictive diagnostics

To assess more rigorously how well the model explained both the biomass and the age composition data, posterior predictive analyses were performed (Gelman et al. 1995). In such analyses one simulates observations from the likelihood (given the posterior sample), and using some suitable summary statistic relating to variation one compares the probability with which the variance in the data as simulated from the model exceeds the observed variance in the data themselves. Doing this one obtains a Bayesian p value (Meng 1994; Gelman et al. 1995) with a value of 0.5, suggesting the model is explaining the data well (although one should plot the distributions of values, as they need not be the same even with a p value of 0.5); values less than or greater than 0.5 suggest that the model predictions are less or more variable, respec-

Table 3. Posterior model probabilities for model *m*, expressed in terms of the minimum age class in the separable age set, A_s.

	$m = \min(A_s)$							
	1	2	3	4	5	6	7	8
$P(m)$	5.8×10^{-8}	3.9×10^{-6}	2.9×10^{-3}	0.0097	0.032	0.28	0.33	0.34

tively, than the data. There is no single statistic than must be used — Gelman et al. (1995) suggest using a χ^2 discrepancy statistic — but in this paper I use the median absolute deviation to derive the Bayesian *p* values. The main reason is that such statistics are (*i*) ideal for deriving such Bayesian *p* values, (*ii*) just as simple to compute as the χ^2 discrepancy statistic, and (*iii*) are used in robust regression techniques and provide a robust alternative to χ^2 approaches for non-normal likelihoods (Hillary 2011).

For the biomass data a posterior predictive analysis was performed, but this is somewhat more difficult for age type data as it requires a univariate discrepancy statistic, but one has multivariate predictions. I will briefly outline a method for obtaining such a univariate discrepancy statistic for use in Bayesian analyses where age type data are used. Given our assumed multinomial distribution for the survey data-at-age as defined in eq. 7, one can simulate the survey data-at-age, $\widetilde{I}_{y,a}$, given the posterior sample, and one can compute the following paired sample of residuals:

$$(26)\qquad \begin{aligned} \varepsilon^1_{y,a} &= \widetilde{I}_{y,a} - \widehat{I}_{y,a} \\ \varepsilon^2_{y,a} &= I_{y,a} - \widehat{I}_{y,a} \end{aligned}$$

In eq. 27 the superscripts 1 and 2 represent the residuals relating to the simulated data's deviation from the model and the true data's deviation from the model, respectively. One then computes the following weighted residuals based on this paired sample of age-specific residuals:

$$(27)\qquad \begin{aligned} \delta^1_y &= \sum_a \varepsilon^1_{y,a}\widehat{p}_{y,a} \\ \delta^2_y &= \sum_a \varepsilon^2_{y,a}\widehat{p}_{y,a} \end{aligned}$$

where $\widehat{p}_{y,a}$ are the model-predicted survey proportions-at-age. The observed and predicted discrepancy statistics are defined as

$$(28)\qquad \begin{aligned} \Delta^1 &= \mathrm{median}|\delta^1_y - \mathrm{median}(\delta^1_y)| \\ \Delta^2 &= \mathrm{median}|\delta^2_y - \mathrm{median}(\delta^2_y)| \end{aligned}$$

The relevant Bayesian *p* value is simply defined as $p(\Delta^1 > \Delta^2)$.

The MCMC samplers were written in C++ but linked into the R statistical language framework. The exploratory data analyses, statistical postprocessing of the MCMC population variables and parameters, and graphics were done using the FLR framework (Kell et al. 2007), a fisheries-specific platform in the R statistical language. All the herring biological and survey data contained in the relevant FLR objects are passed to the MCMC routines via the linked C++ code. In terms of code run times, the model-exploration RJMCMC routine took around 4 h to converge on the posterior, but the model chosen as most appropriate (see later; 131 parameters) converged in around 40 min on a standard laptop.

Results

Selecting the most appropriate mortality model

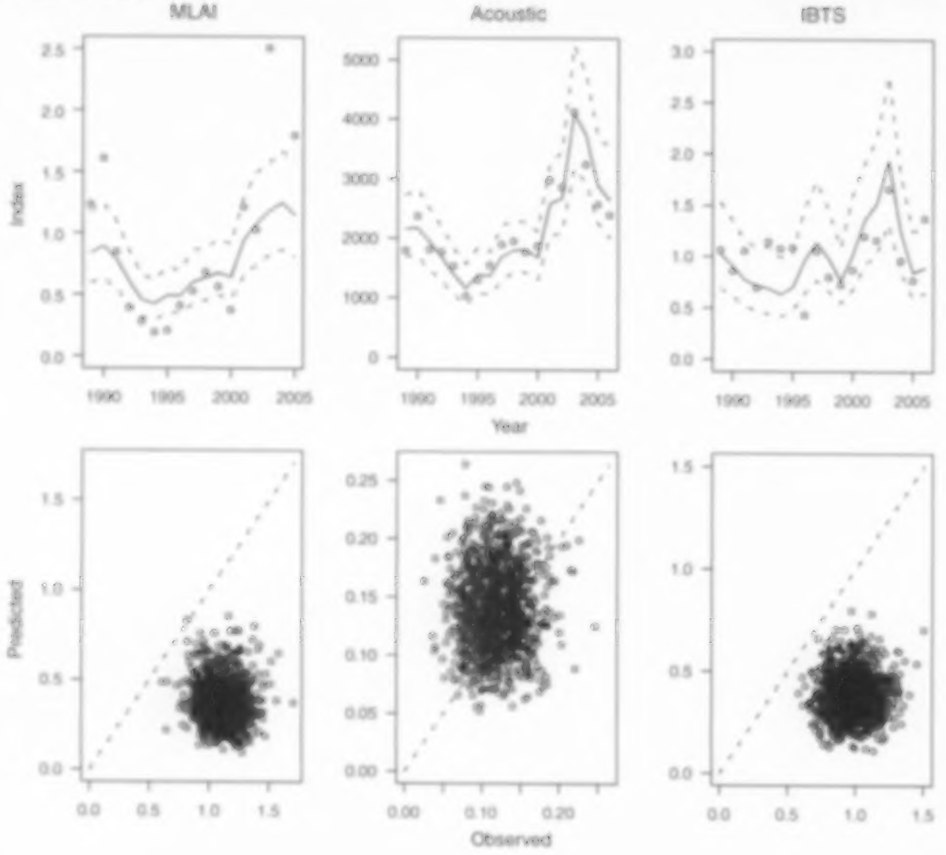
The posterior model probabilities are detailed (Table 3), where the model *m* denotes the minimum age class in the set of separable age classes, A_s. Over 95% of the posterior probability mass is attributable to the models that, for ages 5 and under, have a nonseparable model for the survival probabilities — a Bayes factor of about 21 when comparing these models with all the others. The data overwhelmingly supported models where up to and including at least age 5, survival probabilities could not be explained by a single year effect and a fixed age effect. In terms of Bayes' factors, the evidence for a totally separable survival model versus the evidence for a separable model for ages 6 and above only is decisive (>100). For comparing among the three most sampled models (a separable model for ages 6 to 8, for ages 7 and 8 only, and a totally unseparable model), there was no clear evidence for one model to be more plausible than the other — from Table 3 there is barely any increase in model probability as the model complexity increases. In terms of model choice, given the results of the RJMCMC routine runs and the resultant Bayes' factor analyses, I decided to proceed using the most likely yet most parsimonious of the candidate models where a separable survival model is assumed for ages 6 to 8 **only**. The issue of potential overfitting of the chosen model will be addressed from the standpoint of data weighting in the following subsection.

Data explanation and population trends

For the model assuming a separable survival model for ages 6 to 8 only, I present the population trends (recruitment, survival, and mature biomass) as well as the performance of the model in terms of explaining the survey data. I also compare the results from this work and the current stock assessment that integrates the survey and the catch data in a tuned-VPA algorithm. For this model there are 131 estimated parameters. For clarity, I use the term "fitted" with regards to a model and "data" to mean how closely the model fits to the observed data; when using the term "explain" I mean something stronger in that it is not just how well the data are fitted by the model but how well the variance in the data are explained in terms of the assumptions made when defining the likelihood.

In terms of the posterior predictive analyses for the biomass indices, for the MLAI survey the *p* value was 0.001, for the acoustic biomass the *p* value was 0.686, and for the IBTS biomass the *p* value was 0.002. Looking at the actual fits to the data (Fig. 3), one observes a poor fit to the MLAI survey data was obtained where neither the strength of de-

Fig. 3. (Top panels) The fits (median full line with dotted line 95% credible interval) to the biomass indices for the MLAI (left column), acoustic (centre column) and IBTS (right column) surveys. (Bottom panels) The associated probability plots for the discrepancy statistics used in the posterior predictive analysis.

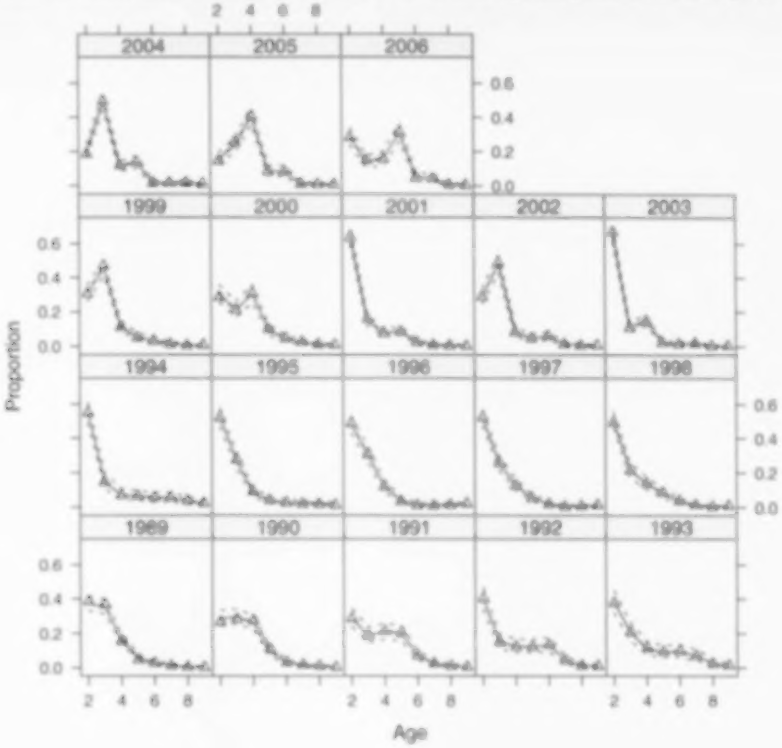


cline observed in the 1990s nor the strength of the resultant recovery in the early 2000s can be reproduced by the model. Perhaps not surprisingly a large process error standard deviation (posterior mean of 0.49) was estimated for these data. This is strongly suggestive of a poor fit (large amounts of extra variance required to explain the data), but the *p* value of 0.001 and the associated plot in Fig. 3 suggest something stronger in that one can also assess that perhaps the choice of likelihood is not ideal — even when actively estimating process error one is still explaining the variational structure in the data poorly. The acoustic data are fitted well with negligible levels of process error estimated, and looking at the posterior predictive plot (along with the *p* value) these data are explained much better by the model than the MLAI or the IBTS data, although given a *p* value > 0.5 it appears the predicted data are somewhat more variable than the actual data. For the IBTS data, while the data are not fitted well overall, the general trend is fitted much better than for the MLAI survey. In terms of the posterior predictive results, a very small *p* value (0.002) is obtained, but the estimates of process error were very small. Additional variance within the model may not be needed to explain the total variance in the data, but in distributional terms the assumed likelihood did not explain the variational structure particularly well at all. In general, a posterior predictive analysis can tell us much more than a simple visual inspection of the fits to the data and estimates of process error, as it can show where distributional assumptions may not be being met.

It comes as no major surprise that the acoustic data are explained the best — they are accurate (in terms of observation error), informative, and consistent (trends in survival and biomass are complementary). As seen in the exploratory data analysis, the acoustic and IBTS surveys agree well in terms of overall trend, and it is these data that are fitted the best, but it is also true that the age-composition data for these surveys aid in this process given the consistency between the survival trends in these data and the associated biomass data. The IBTS biomass data are less precise and cover a smaller age range and, as such, do not have so much influence via the likelihood. Given the high estimated process error for the MLAI survey — which could be indicative of a problem with the assumption that relative egg production and SSB are equivalent — these data have the lowest impact on the resultant population trends.

With regards to the age-composition data, the fits to the acoustic data are shown (Fig. 4), with the model predictions

Fig. 4. Fits (median full line with dotted line 95% credible interval) to the acoustic age-composition data (triangles).



being posterior median and 95% credible intervals, and clearly the model fits to these data very well. The same plot is shown for the IBTS data (Fig. 5), which are fitted well, but not as well as the acoustic data, with some clear years and ages where the model and the data diverge. The *p* values for the acoustic and IBTS age data were 0.427 and 0.251, respectively, and the associated plots of the discrepancy statistics are also shown (Fig. 6). From this one can deduce that the model is explaining the acoustic age data well and the IBTS data reasonably well, but in the latter case the predicted data seem less variable than the observed data.

In this framework I assumed a multinomial–Dirichlet probability model for the age composition data, and this permits us to account for variance above or below that normally observed for a pure multinomial process: dispersion. To look at appropriate levels of the dispersion parameter ω, let us first consider the standardized residuals-at-age:

$$(29)\qquad \bar{z}_{y,a}=\frac{N_y(p_{y,a}-\widehat{p}_{y,a})}{\sqrt{\varphi N_y\widehat{p}_{y,a}(1-\widehat{p}_{y,a})}}$$

and φ is as defined in eq. 14. If the assumptions of a multinomial probability model were true, then for $\varphi = 1$ the variance (over the ages) in these residuals would be equal to 1 in all years. The degree to which φ must be scaled to standardize the residuals is the level of dispersion (uniquely defined by the ω and sample sizes). Commonly this value is greater than 1 (i.e., the data are overdispersed), and we are giving too much weight to the data, but values less than 1 can be indicative of overfitting. This is not conclusive evidence, as it might be that our assumption of a multinomial model was simply wrong and the data follow another distribution, and in practice one tends not to increase the weight of the data based on such an analysis. For the acoustic data, the mean (and CV) in the MCMC estimates of φ were 1.07 (0.13) and for the IBTS data 0.762 (0.19), suggesting that conditional on the multinomial assumption and the observation error CVs, the acoustic data are correctly weighted, but that the model is fitting the IBTS data better than one would expect and, as such, possibly overfitting these data. This, however, conflicts with the *p* value of 0.25,7 suggesting that the data simulated from the model are less "extreme" than the actual data themselves; the (probability) model is not explaining the IBTS data as well as the acoustic data. There is no theoretical equivalence between the posterior predictive approach and the analysis of standardized residuals using the formula in eq. 29 — the former is semiparametric while the latter is fully parametric. It is useful to look at multiple approaches in regard to data weighting in integrated assessments even if they may in fact indicate contradictory things. For both the acoustic and IBTS surveys, no reweighting of the data was performed and the original runs were retained as the final runs — even with conflicting information with respect to the IBTS data, neither approach indicated anything seriously wrong. These results do not suggest any substantial levels of overfitting to the age-composition data even though they are being explained well — especially the acoustic data. Given

Fig. 5. Fits (median full line with dotted line 95% credible interval) to the IBTS age-composition data (triangles).

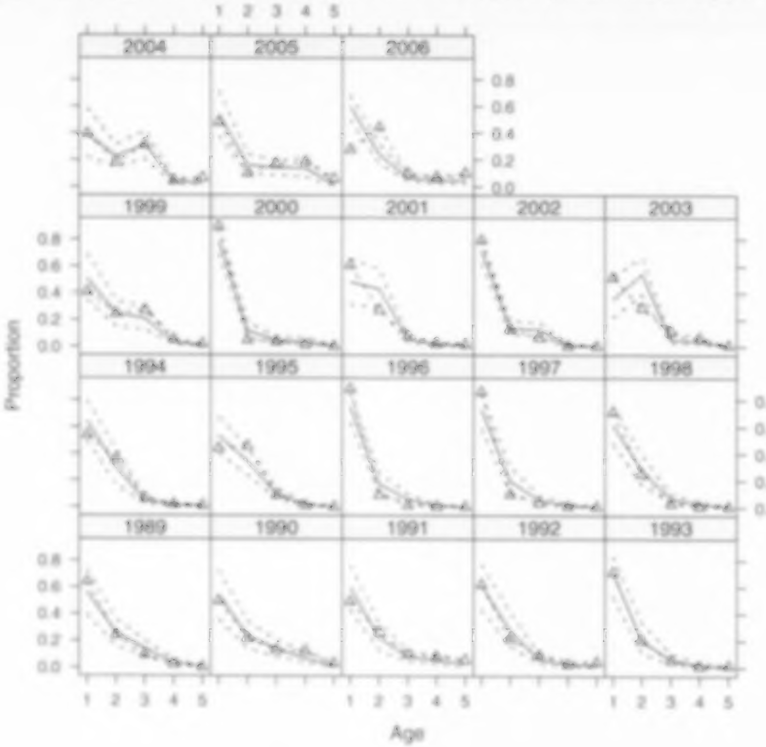
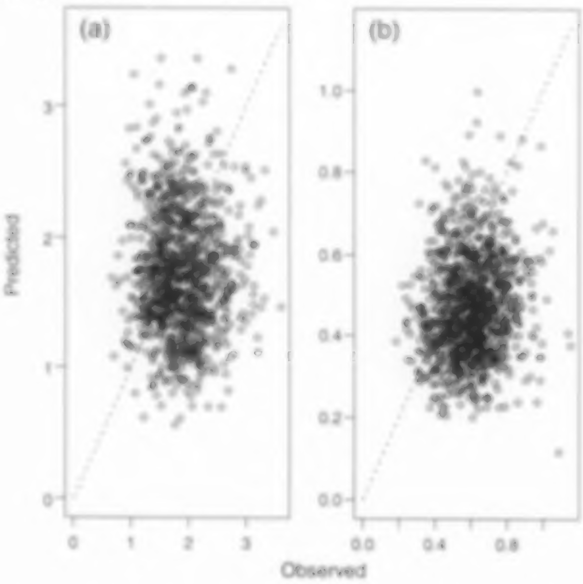


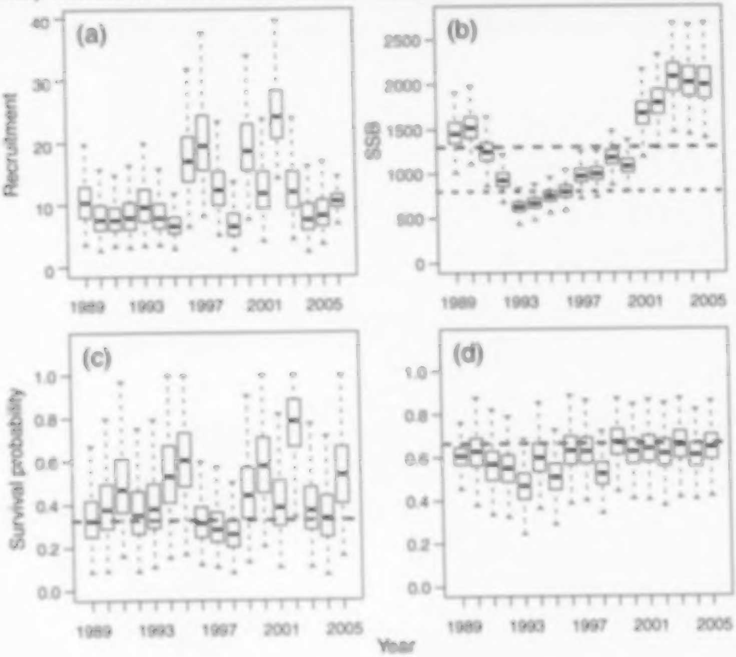
Fig. 6. Probability plots for the discrepancy statistics used in the posterior predictive analysis of the (*a*) acoustic and (*b*) IBTS age composition data.



the precision of the acoustic data (Tables 1 and 2), we should expect to be fitting them well if the model has the freedom to do so, which it does given the flexible survival model.

A summary of the key population variables derived from parameters and process variables estimated in the model is shown (Fig. 7). In terms of reference points (which drive the North Sea herring harvest control rule), there are precautionary and limit SSB levels (1.3 and 0.8 million tonnes, respectively) and two precautionary levels of fishing mortality (International Council for the Exploration of the Sea 2008) for juveniles (ages 0–1: 0.12) and adults (ages 2–6: 0.25). This model does not estimate fishing mortality but total survival probability, and using the values of natural mortality estimated for this stock (International Council for the Exploration of the Sea 2008) I derived associated precautionary survival levels for juveniles (age 1 (0.326) only here as I do not have data covering age 0) and adults (ages 2–6: 0.663) so that $\pi^{t}_{\mathrm{PA}} = \exp(-F_{\mathrm{PA}} - M)$. (In Fig. 7 all longer dashed horizontal lines refer to precautionary reference levels, and the shorter dashed lines refer to limit reference levels.) In terms of recruitment trends, while there have been episodic large recruitment levels in the late 1990s and early 2000s recent recruitments have been at or below mean recent levels. The SSB decreased below limit levels in the early 1990s, with markedly lower survival of the mature population in these years (Fig. 7*d*). However, the better than average survival of juveniles from 1993 to 1995 (Fig. 7*c*), increasing recruits in 1996 and 1997 and increasing survival of mature animals begins to rebuild the SSB above limit levels. SSB stayed below precautionary levels for the rest of the decade, however, until a combination of higher recruitments with higher survival probabilities in 2000–2002 causes the SSB to rise above the precautionary levels in 2001, where it has

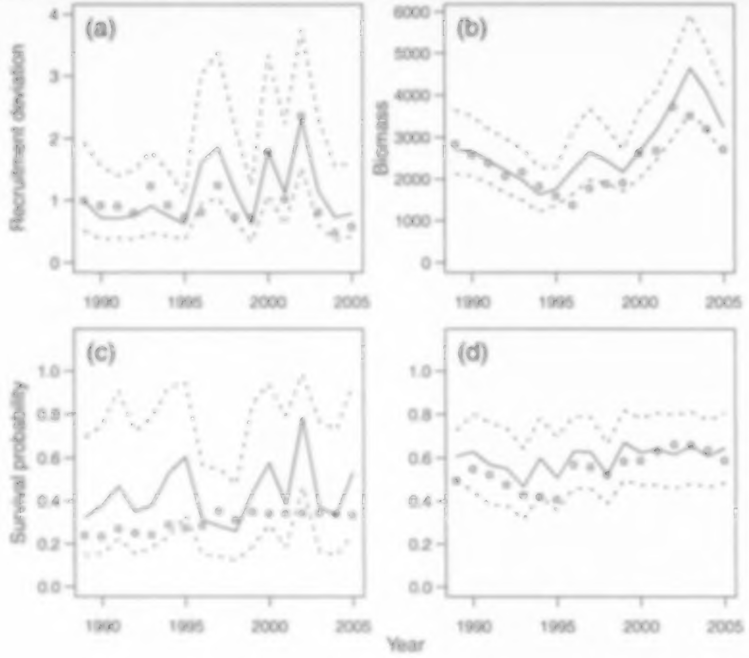
Fig. 7. Summary of the recruitment (millions) (*a*), spawning biomass (×10^3 tonnes) (*b*), and juvenile (*c*) and adult (*d*) survival probabilities estimated using the survey-based assessment model for the most probable model as detailed in the model exploration section. Longer dashed lines correspond to precautionary reference levels; shorter dashed lines correspond to limit reference levels.



stayed considerably above since 2002 until 2005. In terms of general survival trends for the juveniles, only in 1998 was there a reasonable probability (about 0.75) that survival was below precautionary levels, with years in the late 1990s and early 2000s with substantially higher levels of juvenile survival. For adult survival, this shows a more stable trend (median values between about 0.45 and 0.6) but shows a noticeable decrease seemingly commensurate with the SSB crash in the early 1990s.

One very useful comparison is between the trends in recruitment, stock biomass, and survival estimated using the survey data-only model versus those estimated from the current stock assessment. North Sea herring are assessed using a tuned VPA algorithm called ICA (integrated catch analysis; Patterson and Melvin 1996), which uses the same survey data used here (although in a different format for the age data, and the acoustic data are not treated as an absolute abundance index) along with a 0-group survey (so ages covered are 0–9) and the catch-at-age data (from ages 0–9). The data are weighted in a modified least squares framework, with catch data able to be down-weighted but still influential in terms of absolute abundance and fishing mortality, and a separable model for fishing mortality is assumed for all ages for the final 5 years of the data. Figure 8 shows the comparison of the two models' population trends, with ICA-predicted survival probability calculated as $\exp(-F - M)$. Concentrating on recruitment trends assuming recruitment at age 1 (ICA estimates recruitment at age 0 but given a value of $M_0 = 1$, this dominates the estimates of F at age 0 not unduly affecting recruitment trend comparison), one sees a very good agreement between the two models. Only once does an ICA estimate sit outside the 95% credible interval of the survey-based model, and the only apparent consistent divergence occurs where the survey-based estimates are higher than ICA from 2002 to 2005. In terms of total stock biomass (expressed in absolute terms in Fig. 8), the two models agree very closely from 1989 to 1995 until the survey-based estimate begins to recover faster than is predicted by ICA. The two biomass levels converge again in 1999–2000 and remain close until 2002, where the survey-based estimates rise for one further year before declining from 2003 to 2005 with the exact same trend as that estimated by ICA, albeit at a significantly (probability > 0.95) higher level. With respect to juvenile survival, the survey assessment predicts higher levels of survival from 1989 to around 1994 (though not significantly so) before converging to similar levels in the late 1990s and then returning to levels greater than (though significantly so only in 2002) or equal to (once in 2004) those predicted by ICA. Note the high uncertainty in the survey-based estimates of juvenile survival given the IBTS data (with low sample sizes) are the only data that fully cover this age range. For adult survival one observes a much more consistent picture with very similar levels of survival estimates — especially from 1996 onwards — and not once is the ICA estimate outside the 95% credible interval of the survey-based estimates. It is the differences in survival trend that largely drive the observed differences in stock biomass between the two models. The noticeably higher survival estimates of juveniles and adults in 1994 and 1995, as well as some higher recruitment estimates in 1996–1998, seen in the survey-based results cause the more rapid recovery of the stock biomass, relative to the ICA estimate. The second bio-

Fig. 8. Comparison between survey-based assessment model (median, full lines; 95% credible interval, dashed lines) and current stock assessment model (points) for recruitment trend (*a*), stock biomass ($\times 10^3$ tonnes) (*b*), and juvenile (*c*) and adult (*d*) survival probabilities.



mass divergence seen in 2003 is caused by a much higher estimate of juvenile survival in 2002.

Overall the two methods agree quite closely in many areas, which is not surprising given they both use largely the same data. The points at which they diverge are driven mostly by differences in survival rates — especially juvenile ones — although smaller recruitment differences are also a factor. This is interesting as the models for survival assumed in the two models are the key difference between them; the population models are practically identical but ICA uses the catch data and an assumed vector of natural mortality-at-age (fixed over years) to separate the two sources of mortality. North Sea herring are a major prey item for various predators, the abundance of which in certain cases has decreased dramatically over the period covered by the data. They are known to exhibit variable growth over time and show sensitivity to various environmental drivers, as well as abundance varying spatially with time (International Council for the Exploration of the Sea 2008). Given the arguments in the Introduction, this suggests that North Sea herring are unlikely to have had a fixed level of natural mortality-at-age across years, and when free from the imposition of separability, the estimates of fishing mortality show little evidence of such a property (International Council for the Exploration of the Sea 2008). The data definitely support the hypothesis that survival-at-age by year — in particular at the youngest ages — varies annually, strongly, and not in a fashion that can be explained by separate year and age effects (see Fig. 9). It is beyond the data to tell us whether this occurs naturally or is due to fishing effects or (most likely) some combination of the two. While the survey-based model is conditioned without catch data, this does not mean that we cannot say anything about exploitation rates given absolute abundance is estimated in the model. A very simple empirical harvest rate can be constructed using the numbers-at-age and the commercial catch-at-age data, $C_{y,a}$:

$$\xi_{y,c}^{\mathrm{surv}} = \min\left(\frac{C_{y,a}}{N_{y,a}}, 1\right) \tag{30}$$

The estimates from ICA can be derived using the Baranov equation:

$$\xi_{y,a}^{\mathrm{ICA}} = \frac{F_{y,a}}{F_{y,a} + M_a}\left[1 - \exp\left(-F_{y,a} - M_a\right)\right] \tag{31}$$

By generating the mean juvenile (age 1) and adult (ages 2 to 6) ranges we can compare the estimates (Fig. 10). Furthermore one can in principle from this empirical estimate of harvest rate produce an estimate of natural survival as

$$\pi_{y,a}^{s} = \pi_{y,a}^{m}\left(1 - \xi_{y,a}^{\mathrm{surv}}\right) \tag{32}$$

So, by expressing natural mortality as $-\log\left(\pi_{y,\bullet}^{m}\right)$, one can compare the empirical estimates with those assumed in the stock assessment (Fig. 11). Clearly the empirical estimates of natural mortality for the juvenile (age 1) and adult (ages 2–6) portions of the stock are highly variable (Fig. 11), and one must not forget that I assumed the catch-at-age data to be known without error in eq. 30, so this may not be the full extent of the variance. Conversely, the empirical estimates of adult *M* are averaged over ages 2 to 6, so if they vary with trend relative to each other this will tend to inflate the uncertainty in the resultant estimates. If one assumes that the uncertainty in the estimates of *M* is dominated by uncertainty

Fig. 9. Survival probability by year and age for the totally nonseparable model and with the highest posterior probability mass (see Table 3).

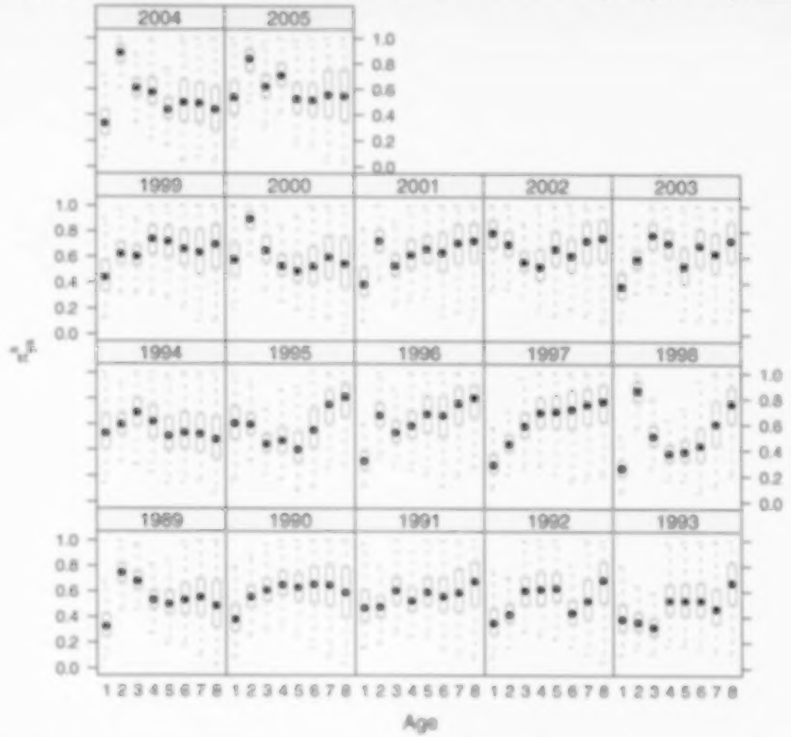


Fig. 10. Juvenile (*a*) and adult (*b*) empirical exploitation rates using the catch-at-age data and the numbers-at-age from the survey-based assessment (median, solid lines; 95% credible interval, dashed lines) versus those predicted by the current assessment (circles).

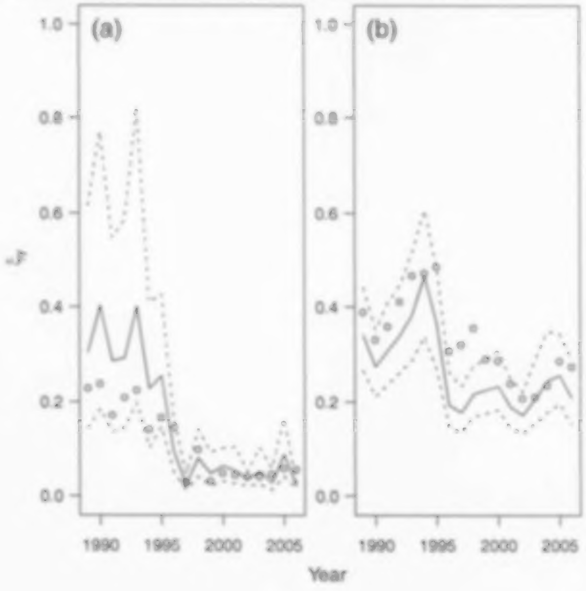
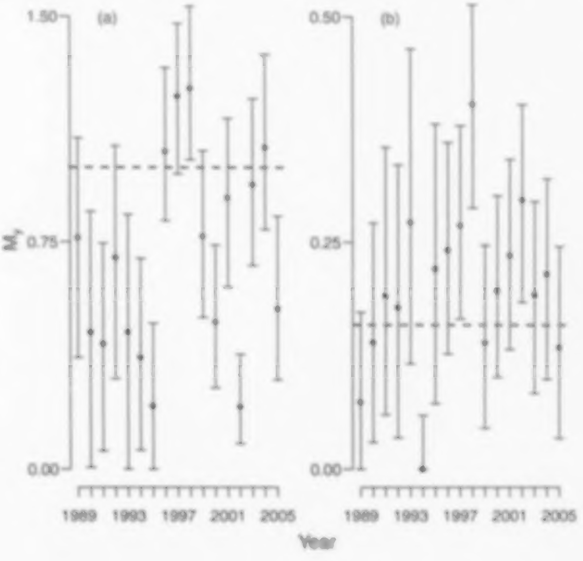


Fig. 11. Juvenile (*a*) and adult (*b*) empirical estimates (circles are the posterior median with the whiskers covering the interquartile range) of natural mortality-at-age by year for North Sea herring conditional on the exploitation rate estimates in Figure 9. The broken lines represent the values assumed in the stock assessment model.



in abundance and survival-at-age, then we can perhaps conclude that there is evidence that M may be varying with time and not in a random fashion. For the juvenile animals, median estimates of M are repeatedly below the assessment-assumed level of 1 often with probability greater than 0.75. Averaged over years, $M_1 = 0.73$ and $p(M_1 < 1) = 0.967$. For the adult portion of the stock, the converse is true, with the mean level of M consistently higher than the assessment level of 0.16, although there are only two instances when this occurs with probability greater than 0.75. Averaged over years $M_{2\text{–}6} = 0.22$ and $p(M_{2\text{–}6} > 0.16) = 0.992$.

The stock assessment estimates of M were derived from the multispecies VPA work done by Helgason and Gislason (1979) and Pope (1979), but the more recent similar analyses undertaken by Vinther (2001) show (at least for the juvenile ages for which there was data) that estimates of M for age 1 North Sea herring appear to have gradually decreased by almost a factor of a half from around 1975 to 1998 and to levels of around 0.4 to 0.75. This trend roughly agrees (up to 1995) with the estimates obtained in this work. Given the strong decrease in abundance of many of the predators of juvenile herring over the period from 1975 to the present, it is perhaps not so surprising to observe such a decrease in juvenile natural mortality. With respect to the level of natural mortality on the mature animals (ages 2 to 6) appearing somewhat higher than assumed before, there is also a potential explanation; mean mass-at-age for these age classes has decreased by about 10% from levels seen in the 1960–1980 period to those seen in the years covered by the data used in the survey-based assessment (International Council for the Exploration of the Sea 2008). If this results in a decrease in the mean length-at-age (i.e., mass-at-length relationship has not changed appreciably), then on average, animals aged 2–6 now are shorter (by around 10%) than they were when the assessment estimates of M were formulated. If we assume changes in natural mortality are governed by length (longer animals have lower values of M — excluding senescent length classes where it increases), then one might expect shorter animals of the same age to experience higher levels of natural mortality, which is perhaps what is being observed here.

In terms of the apparent structure of the natural mortality-at-age vector, at least averaged over the years covered in these analyses, there are two general observations: (*i*) M appears much higher for age 1 fish than all other ages (see previous paragraph), which is also assumed in the stock assessment M vector (International Council for the Exploration of the Sea 2008). (*ii*) It does not appear to fit into standard forms, such as those explored in Lorenzen (1996), where natural mortality was expressed as a monotonically decreasing power function of age–length–mass. For the herring estimates derived in this work, and in the multispecies VPA work done in the past, they do appear to rapidly decrease from age 1 to ages 2 and 3 at a rate much faster than that estimated from an array of oceanic and aquaculture data in Lorenzen (1996). What is potentially interesting is that while more uncertain at the older ages, they do seem to show a subsequent increase in the older ages of 6, 7, and 8, giving the M vector an asymmetric U-shape that potentially indicates some senescent effects.

Managing without fishing mortality

One obvious and immediate criticism is what can be done with total mortality or survival probabilities alone? Fisheries impact is traditionally associated (in the single species sense at least) with the levels of fishing mortality and exploitation rate being felt by the population. Irrespective of whether input (effort–capacity regulation, closed areas, and (or) seasons), output (catch–bycatch levels), or both input and output controls are used in terms of managing the fishery, the fishing mortality almost is always used as an indicator of stock status (relative to F-based reference points, for example). The EU FP6 project FISBOAT was concerned with investigating how survey-based methods might perform in fisheries stock assessment and management, and several papers derived from this work looked at how total mortality and survey abundance-based harvest control rules might be constructed (Apostolaki and Hillary 2009; Hillary 2009) and how they performed for a variety of EU stocks (including North Sea herring) using management strategy evaluation (Pomarede et al. 2010). The rules based on total mortality and (or) survey abundance were either ad hoc in nature (adapting the TAC based upon trends in Z over time) or employed Z-based reference points generalized from their F-based counterparts and natural mortality (as done in this paper).

The notion of Z-based reference points is by no means new (Caddy and Csirke 1983), where the notion of maximum biological and surplus production and not maximum sustainable yield was the key paradigm. The main idea is that there is some level of total mortality where the biomass due to incoming recruitment and stock growth minus that lost to mortality is at its greatest. Clearly, without a priori knowledge of the stock–recruit relationship, this concept can work only on a per-recruit basis. With the type of outputs available in the assessment model detailed in this paper, one can quite naturally derive $F_{\max}$ and $F_{0.1}$ proxies, but in terms of survival probabilities. Given some "reference" survival vector $\tilde{\pi}^s_a$, one can derive the survival age effect as follows: the age at which $\tilde{\pi}^s_a$ is highest has an age effect (in logit space) of zero, and the rest follow from this value with the condition of equality in terms of the survival proportions-at-age. This is all done in logit space to ensure none of the problems with a "separable" multiplicative survival model detailed previously occur. Lastly one must choose some "reference" value of the survival year effect (magnitude), $\tilde{\eta}$, which could correspond to a level of survival expected in the absence of fishing (based on some "average" estimate of M) or perhaps some "maximal" survival regime. In a per-recruit setting the equilibrium age structure is defined to be

$$(33)\qquad N_a = \prod_{i=1}^{a-1} [1 + \exp(-\eta - \chi_i)]^{-1}$$

The associated biomass per recruit at the start of the year is

$$(34)\qquad B^p = \sum_{a=1}^{A} \left\{ \prod_{i=1}^{a-1} [1 + \exp(-\eta - \chi_i)]^{-1} \right\} w_a$$

The biomass expected at the end of the year assuming the reference level of survival probability would be

$$(35)\qquad B^{w} = w_1 + \sum_{a=1}^{A-1}\left\{\prod_{i=1}^{a-1}\left[1+\exp\left(-\eta-\chi_i\right)\right]^{-1}\right\} \times\left[1+\exp\left(-\tilde{\eta}-\chi_a\right)\right]^{-1} w_{a+1}$$

The w_1 value represents the biomass addition of a single recruit (given the per-recruit nature of the analysis). The biological net production of biomass in a given year (per recruit) is then simply defined:

$$(36)\qquad \mathrm{BSP} = B^{w'} - B^{w} = g(\eta)$$

The $F_{\max}$ proxy is the value of η that maximizes the biological (net) production of biomass in eq. 36:

$$(37)\qquad \eta_{\mathrm{MBP}} = \arg\max_{\eta}\, g(\eta)$$

By definition $g(\tilde{\eta}) = 0$ and the $F_{0.1}$ proxy follows as one would expect as being the value of η that results in a gradient of BSP equal to 10% of that seen at $\eta = \tilde{\eta}$:

$$(38)\qquad \eta_{0.1} = \eta^{*}\, s.t.\frac{\mathrm{d}g}{\mathrm{d}\eta}(\eta^{*}) = 0.1\,\frac{\mathrm{d}g}{\mathrm{d}\eta}(\tilde{\eta})$$

For concave $g(\eta)$, then $\eta_{0.1}$ will be less than η_{MBP} (as with $F_{\max}$ and $F_{0.1}$). Assuming a maximum survival probability of 0.95 to define the reference level $\tilde{\eta}$ and using the most recent year (2006) to define the age effect, χ_a, the posterior (median and 95th percentile), survival probability multipliers for the two surplus production-based reference points η_{MBP} and $\eta_{0.1}$ were 0.65 (0.18–0.7) and 0.67 (0.44–0.72). One can also compute the spawning biomass-per-recruit reduction ratio (Mace and Sissenwine 1993), relative to $\tilde{\eta}$, as another reference point and also as a check on the sensibility (or otherwise) of the derived maximum biological production reference points. If one had information on the stock–recruit relationship — in particular how recruitment levels change relative to reference SSB levels as survival decreases — one can extend the reference points to an MSY proxy based on equilibrium surplus biomass production. It should be stated that one can expect that all the problems with, and objections to, such per-recruit reference points as $F_{\max}$ and $F_{0.1}$ also apply to η_{MBP} and $\eta_{0.1}$. All reference points have elements of subjectivity and flaws in derivation assumptions, and the intension was not to provide alternative total mortality target points — perhaps their best function might be as precautionary ($\eta_{0.1}$) and limit (η_{MBP}) reference points — but a way of deriving reference points when using such an assessment method.

Discussion

Using survey data as the sole basis of stock assessment has become increasingly popular (Beare et al. 2005; Porch et al. 2006; Cotter et al. 2007) primarily because of issues with the commercial data or where the parameters required for a more standard assessment type model are unknown. There are also strong arguments in favour of using only survey data as the tuning data (Ibaibarriaga et al. 2008) even in the presence of accurate catch and effort data given both the explicit design of surveys (and most importantly what they observe) and the frequent difficulty of interpretation of commercial data-derived abundance trends (Harley et al. 2001; Polacheck 2006). Although, as pointed out in Wilberg and Bence (2006), this still requires "good" (reasonably precise and abundance-related) survey data and depends on the specific issue with the fishery-dependent data.

Survey-based data have the potential to provide us with a large array of information useful for managing fish stocks (Cotter 2009; Petitgas et al. 2009), and in the work of Pomarede et al. (2010) it was demonstrated how trends in mature biomass, recruitment, and total mortality derived directly from survey data can be used to construct survey-based, catch-free management procedures that are robust to a variety of key uncertainties — in particular under-reporting or biased catch data and changes in natural mortality over time.

In this work, survey data are the primary data sources, but there are other forms of potentially useful assessment data unrelated to commercial operations. Mark–recapture data in the capture fishery arena is traditionally related to the fishery in some manner, the usual form being that tags are released into the population and the fishery acts as the population resampler with recaptures from the fishery being used in a variety of ways to make inference about abundance and mortality (Seber 1982; Polacheck et al. 2006). Skaug (2001) proposed a genetic approach where samples from the juvenile and adult portions of the population are genetically profiled, and the information on parent–child matches can be used to estimate absolute adult abundance; a similar approach (Bravington and Grewe 2007) is being developed for application to southern bluefin tuna (*Thunnus maccoyii*). Genetic tagging — both the "close-kin" (Skaug 2001; Bravington and Grewe 2007) and "standard" mark–recapture approaches — have the potential to become highly informative data types in the near future.

In this paper I have detailed a Bayesian survey, data-based and catch-free (in terms of estimating the key parameter estimates), assessment model using North Sea herring as the test stock and data set. The model is able to integrate age-based (or length–stage data if applicable) and biomass data with a flexible model for survival probabilities, not total mortality, allowing years and age classes where survival separates (consistently) into year and age effects, which also permits the inclusion of mortality covariates in a consistent manner. Key parameters estimated are survival probabilities, mature biomass, and recruitment trends over time. In this particular case absolute biomass was estimated, assuming the North Sea herring acoustic biomass data to be absolute, and it was observed that the catch-free assessment estimated very similar recruitment trends and absolute biomass levels as the current International Council for the Exploration of the Sea stock assessment, with differences driven largely by different estimates of survival between the two models at certain periods in the data coverage (ages 1–9 and 1989–2006). Using powerful MCMC techniques, an exploration of the model space, relating to the separation of mortality into year and age effects, was undertaken, and this showed quite clearly that the data supports the hypothesis that survival probabilities for North Sea herring over both juvenile and adult key age ranges (i.e., up to age 6) cannot be easily separated into age and year effects — an assumption common to most survey-based assessment models that do not estimate fishing mortality (Beare et al. 2005; Needle 2005). While not prima facie evidence for changes in natural mortality, over time it does suggest quite strongly that either natural mortality, fishing mortality, or both display nonseparable dynamics over the data coverage.

Using the catch-at-age data to derive empirical exploitation rates, estimates of juvenile (age 1) and adult (ages 2 to 6) natural mortality by year were obtained and compared with the fixed levels assumed in the stock assessment. In line with the results of Vinther (2001), levels of natural mortality for the juveniles were mostly lower than the values computed in the multispecies VPA work of Helgason and Gislason (1979) and Pope (1979). For the adults, mortality levels were estimated to be mostly higher than the currently assumed values. For the juveniles this makes sense given the large declines in predator abundance observed over the relevant time frame, and for the adults there is a potential explanation given the decrease in mean length-at-age observed in the past two decades.

It was shown how one can derive survival-based reference points comparable to $F_{\max}$ and $F_{0.1}$, given the outputs of the assessment model detailed herein, but their potential usage will very likely have all of the problems associated with their *F*-based counterparts. The main purpose was to show how one can still in theory derive such reference points even when not explicitly estimating fishing mortality. The work done in Apostolaki and Hillary (2009) and Hillary (2009) showed how one can link survey-derived information to actual management quantities (such as catch quotas and (or) fishing effort) via survey-based harvest control rules, and Pomarede et al. (2010) demonstrated that these management strategies are quite robust to issues that fishery-dependent strategies are not. Perhaps the role for the reference points defined here are as constituent parts of these survey-based management strategies or to be used in performance measures when evaluating these strategies, in a management strategy evaluation context.

Any population that is a major prey item for a number of predators whose abundance changes over time, or is strongly influenced by environmental conditions, is likely to experience changes in natural mortality over time. Also, natural mortality that is constant across ages–lengths as well as years is surely going to be the very rare exception and not the rule. In more conventional stock assessment models, natural mortality is almost always fixed over time, and frequently across ages, as we tend to lack the level of information in the available data to detect such changes while estimating other key population variables and separating fishing from natural mortality. Survey-based assessments might have been developed more to avoid issues with using commercial data in stock assessment, but they can also be a tool for detecting and accounting (albeit "blindly") for patterns in both natural and fishing mortality that break the assumptions of conventional stock assessment models. Managing exploited stocks using fishing mortality may be historically common, but that does not mean one cannot follow alternate approaches with perhaps less familiar control variables. In this regard one can consider an assessment such as that detailed in this paper as a biological smoother of sorts, with the population model acting as the smoother, where the harvest control rule is constructed using only survey-derived information (Apostolaki and Hillary 2009).

In stock assessment development there are examples and packages that attempt to condition highly complex population and fishery models, integrating all sources of (primarily commercial) data (precise and imprecise and potentially biased) at once — in particular in the tropical tuna world (Hampton and Fournier 2001). This is in part because of the complexity of the populations and fisheries that exploit them and also because of the lack of fishery-independent data for such stocks. This is a key weakness of models such as the one detailed in this paper — it requires good quality survey data. Surveys are an expensive way of obtaining information on a given population, especially in the presence of fishery-dependent data that has a much lower effective cost (in terms of collection and standardization). Also, there are many stocks for which there will likely never be such survey data — highly migratory pelagic species, for example. Cotter et al. (2004) argued the case for simplicity in stock assessments, making them robust to the assumptions made in more complex models, suggesting that at least for the North Sea stocks discussed, using survey data to estimate trends in total mortality and abundance was preferable. The population and probability models and estimation framework explored in this work likely fails their criteria (Cotter et al. 2004) on at least two fronts (complicated probability model and data weighting framework; use of Bayesian and in particular complex MCMC techniques). However, there is perhaps a case for a middle ground where one attempts to make as few assumptions as possible and where one makes the most out of the best and most trusted data. This might require complex techniques and a decrease in the ability to translate the model to a wider audience of stakeholders to avoid having to make either management decisions based on biased parameters or assumptions that may not be supported by the data.

Acknowledgements

This work was inspired by work done by and conversations with the people who worked on the EU FP6 FISBOAT (Fishery Independent Survey-Based Operational Assessment Tools) — in particular John Cotter and Marine Pomarede. The author also thanks two anonymous referees for their comments and suggestions on a previous version of this manuscript.

References

Apostolaki, T., and Hillary, R.M. 2009. Harvest control rules in the context of fishery-independent management of fish stocks. Aquat. Living Resour. **22**(2): 217–224. doi:10.1051/alr/2009022.

Beare, D.J., Needle, C.J., Burns, F., and Reid, D.G. 2005. Using survey-data independently from commercial data in stock assessment: an example using haddock in ICES Division VIa. ICES J. Mar. Sci. **62**(5): 996–1005. doi:10.1016/j.icesjms.2005.03.003.

Bernardo, J.M. 2003. Bayesian statistics. *In* Encyclopaedia of life support systems (EOLSS): probability and statistics. *Edited by* R. Viertl. Oxford University Press, London, UK.

Bogaards, J.A., Kraak, S.B., and Rijnsdorp, A.D. 2009. Bayesian survey-based assessment of North Sea plaice (*Pleuronectes platessa*): extracting integrated signals from multiple surveys. ICES J. Mar. Sci. **66**(4): 665–679. doi:10.1093/icesjms/fsp038.

Bravington, M., and Grewe, P. 2007. A method for estimating the absolute spawning stock size of SBT, using close-kin genetics. CCSBT-ESC/0709/18. CCSBT, Canberra, Australia.

Caddy, J.F., and Csirke, J. 1983. Approximations to sustainable yield for exploited and unexploited stocks. Oceanogr. Trop. **18**(1): 3–15.

Christensen, V., Walters, C.J., and Pauly, D. 2000. ECOPATH with ECOSIM: a user's guide. October 2000 edition. Fisheries Centre, The University of British Columbia/International Centre for Living Aquatic Resources Management, Vancouver, B.C., Canada/ Penang, Malaysia.

Cook, R.M. 1997. Stock trends in six North Sea stocks as revealed by an analysis of research vessel surveys. ICES J. Mar. Sci. **54**(5): 924–933. doi:10.1006/jmsc.1997.0235.

Cotter, A.J.R. 2009. A selection of nonparametric statistical methods for assessing trends in trawl survey indicators as part of an ecosystem approach to fisheries management (EAFM). Aquat. Living Resour. **22**(2): 173–185. doi:10.1051/alr/2009019.

Cotter, A.J.R., Burt, L., Paxton, C.G.M., Fernandez, C., Buckland, S.T., and Pan, J.-X. 2004. Are stock assessment methods too complicated? Fish Fish. **5**(3): 235–254.

Cotter, A.J.R., Mesnil, B., and Piet, G. 2007. Estimating stock parameters from trawl CPUE-at-age series using year-class curves. ICES J. Mar. Sci. **64**(2): 234–247. doi:10.1093/icesjms/fsl025.

Fulton, E.A., Smith, A.D.M., and Punt, A.E. 2005. Which ecological indicators can robustly detect effects of fishing? ICES J. Mar. Sci. **62**(3): 540–551. doi:10.1016/j.icesjms.2004.12.012.

Gelman, A., Carlin, B.J., Stern, H.S., and Rubin, R.B. 1995. Bayesian data analysis. Chapman and Hall, London, UK.

Gilks, W.R., Richardson, S., and Spiegelhalter, D.J. 1996. Markov chain Monte Carlo in practice. Chapman and Hall, London, UK.

Green, P.J. 1995. Reversible jump Markov chain Monte Carlo computation and Bayesian model determination. Biometrika, **82**(4): 711–732. doi:10.1093/biomet/82.4.711.

Hampton, J., and Fournier, D.A. 2001. A spatially disaggregated, length-based, age-structured population model of yellowfin tuna (*Thunnus albacares*) in the western and central Pacific Ocean. Mar. Freshw. Res. **52**(7): 937–963. doi:10.1071/MF01049.

Harley, S.J., Myers, R.A., and Dunn, A. 2001. Is catch-per-unit-effort proportional to abundance? Can. J. Fish. Aquat. Sci. **58**(9): 1760–1772. doi:10.1139/cjfas-58-9-1760.

Helgason, T., and Gislason, H. 1979. VPA analysis with species interaction due to predation. ICES CM 1979/G: 52.

Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York.

Hillary, R.M. 2009. An introduction to FLR fisheries simulation tools. Aquat. Living Resour. **22**(2): 225–232. doi:10.1051/alr/2009023.

Hillary, R.M. 2011. A new method for estimating growth transition matrices. Biometrics, **67**(1): 76–85. doi:10.1111/j.1541-0420.2010.01411.x. PMID:20374243.

Hillary, R.M., Kirkwood, G.P., and Agnew, D.J. 2006. An assessment of toothfish in subarea 48.3 using CASAL. CCAMLR Sci. **13**: 65–95.

Ibaibarriaga, L., Fernandez, C., Uriarte, A., and Roel, B.A. 2008. A two-stage biomass dynamic model for Bay of Biscay anchovy: a Bayesian approach. ICES J. Mar. Sci. **65**(2): 191–205. doi:10.1093/icesjms/fsn002.

International Council for the Exploration of the Sea. 2007. Planning Group for Herring Surveys. ICES CM 2007/LRC:01: 183.

International Council for the Exploration of the Sea. 2008. Report of the Herring Assessment Working Group for the area south of 62°N. ICES CM 2008/ACOM:02.

Irwin, B.J., Treska, T.J., Rudstam, L.G., Sullivan, P.J., Jackson, J.R., VanDeValk, A.J., and Forney, J.L. 2008. Estimating walleye (*Sander vitreus*) density, gear catchability, and mortality using three fishery-independent data sets for Oneida Lake, New York. Can. J. Fish. Aquat. Sci. **65**(7): 1366–1378. doi:10.1139/F08-062.

Jeffreys, H. 1961. Theory of probability. Oxford University Press, London, UK.

Kass, R.E., and Raftery, A.E. 1995. Bayes factors. J. Am. Stat. Assoc. **90**(430): 773–795. doi:10.2307/2291091.

Kell, L.T., Mosqueira, I., Grosjean, P., Fromentin, J.-M., Garcia, D., Hillary, R., Jardim, E., Mardle, S., Pastoors, M.A., Poos, J.J., Scott, F., and Scott, R.D. 2007. FLR: an open-source framework for the evaluation and development of management strategies. ICES J. Mar. Sci. **64**(4): 640–646. doi:10.1093/icesjms/fsm012.

Lorenzen, K. 1996. The relationship between body weight and natural mortality in juvenile and adult fish: a comparison of natural ecosystems and aquaculture. J. Fish Biol. **49**(4): 627–642. doi:10.1111/j.1095-8649.1996.tb00060.x.

Mace, P.M., and Sissenwine, M.P. 1993. How much spawning per recruit is enough? Can. Spec. Publ. Fish. Aquat. Sci. **120**: 101–118.

Meng, X. 1994. Posterior predictive *p*-values. Ann. Stat. **22**(3): 1142–1160. doi:10.1214/aos/1176325622.

Mesnil, B., Cotter, J., Fryer, R.J., Needle, C.L., and Trenkel, V.M. 2009. A review of fishery-independent assessment models, and initial evaluation based on simulated data. Aquat. Living Resour. **22**(2): 207–216. doi:10.1051/alr/2009003.

Needle, C.J. 2005. SURBA 3.0: technical manual. FRS Marine Laboratory, Aberdeen, UK.

Patterson, K., and Beveridge D. S. 1995. Report of the herring larvae surveys in the North Sea in 1994/1995 and adjacent waters. ICES CM 1995/H:21.

Patterson, K.R., and Melvin, G.D. 1996. Integrated catch-at-age analysis Version 1.2. Scottish Fisheries Research Report. FRS Marine Laboratory, Aberdeen, UK.

Petitgas, P., Cotter, A.J.R., Trenkel, V., and Mesnil, B. 2009. Fish stock assessments using surveys and indicators. Aquat. Living Resour. **22**(2): 119. doi:10.1051/alr/2009014.

Polacheck, T. 2006. Tuna longline catch rates in the Indian Ocean: Did industrial fishing result in a 90% rapid decline in the abundance of large predatory species? Mar. Policy, **30**(5): 470–482. doi:10.1016/j.marpol.2005.06.016.

Polacheck, T., Eveson, J.P., Laslett, G.M., Pollock, K.H., and Hearn, W.S. 2006. Integrating catch-at-age and multiyear tagging data: a combined Brownie and Petersen estimation approach in a fishery context. Can. J. Fish. Aquat. Sci. **63**(3): 534–548. doi:10.1139/f05-232.

Pomarede, M., Hillary, R., Ibaibarriaga, L., Boogards, J., and Apostolaki, P. 2010. Evaluating the performance of survey-based operational management procedures. Aquat. Living Resour. **23**(1): 77–94. doi:10.1051/alr/2010005.

Pope, J. G. 1979. A modified cohort analysis in which constant natural mortality is replaced by estimates of predation levels. ICES CM 1979/H: 16.

Porch, C.E., Eklund, A.-M., and Scott, G.P. 2006. A catch-free stock assessment with application to goliath grouper (*Epinephelus itajara*) off southern Florida. Fish Bull. **104**(1): 89–101.

Quinn, T.J., II, and Deriso, R.B. 1999. Quantitative fish dynamics. Oxford University Press, Oxford, UK.

Seber, G.A.F. 1982. The estimation of animal abundance and related parameters. 2nd ed. Charles Griffin and Co. Ltd., London, UK.

Skaug, H.J. 2001. Allele-sharing methods for estimation of population size. Biometrics, **57**(3): 750–756. PMID:11550924.

Spiegelhalter, P.J., Best, N.G., Carlin, B.P., and van der Linde, A. 2002. Bayesian measures of model complexity and fit. J. R. Stat. Soc. B, **64**(4): 583–639. doi:10.1111/1467-9868.00353.

Trenkel, V.M. 2008. A two-stage biomass random effects model for stock assessment without catches: What can be estimated using only biomass and survey indices? Can. J. Fish. Aquat. Sci. **65**(6): 1024–1035. doi:10.1139/F08-028.

Vinther, M. 2001. *Ad hoc* multispecies VPA tuning applied for Baltic and North Sea fish stocks. ICES J. Mar. Sci. **58**(1): 311–320. doi:10.1006/jmsc.2000.1012.

Watters, G.M., Hinke, J.T., Reid, K., and Hill, S. 2005. A krill-predator-fishery model for evaluating candidate management procedures. WG-EMM-05/13. CCAMLR, Hobart, Australia.

Wilberg, M.J., and Bence, J.R. 2006. Performance of time-varying catchability estimators in statistical catch-at-age analysis. Can. J. Fish. Aquat. Sci. **63**(10): 2275–2285. doi:10.1139/F06-111.

Appendix A

There are two steps required when constructing a reversible jump MCMC (Green 1995) sampler: (*i*) sample the parameters from the posterior of a given model; (*ii*) (reversibly) move between two candidate models m_1 and m_2, all the while ensuring that the full model and parameter posterior is the convergent density of the Markov chains.

Sampling scheme for a given model

For a given model m I use Metropolis-within-Gibbs sampling, which is basically a Gibbs sampler (Geman and Geman 1984) applied one parameter at a time that uses the Metropolis–Hastings (Metropolis et al. 1953; Hastings 1970) algorithm to sample from the conditional posterior of the given parameter if it is not of a known form. The conditional posteriors for the log-scale survey catchability parameters estimated for the IBTS biomass and MLAI survey data are normal eq. 21 and are sampled directly. This is because of the normal-log assumption: the only difference between a random variable X with a normal-log distribution and one with a log-normal distribution is that the $X^{-1/2}$ term in the density is no longer there, as it is in the log-normal density. All the other relevant parameters ($\gamma^s_{y,a}$, η_y, χ_a, ε^r, ε^a, $\ln(\bar{R})$, ψ, σ_p) are sampled using the (random walk with normal proposal distribution) Metropolis–Hastings algorithm. When only one model was selected, convergence of the Markov chains on the posterior was established using standard techniques (Brooks and Roberts 1998), where multiple independent Markov chains are simulated and several inter- and intra-chain statistics are checked to ascertain convergence. A 1000 iteration burn-in period was assumed with 100 000 further samples being generated and where each 100th sample was retained (a thinning factor of 100 to remove autocorrelation), thus resulting in a 1000 samples from the posterior.

Reversible jump scheme to move between models

When constructing a reversible jump MCMC algorithm, especially when there are dimensional changes (in terms of the number of parameters), one has to rigorously define where the sampler may move to and from, the probability of that move, and how to generate proposal moves (i.e., how to generate new parameters when increasing dimension and how to "collapse" the system when decreasing dimension). There are many formulations of the transition acceptance probability associated with the move from models m_1 and m_2, but for the purposes of this discussion, I will make it generic enough to be able to encompass the moves required. As in the Metropolis–Hastings algorithm, the acceptance probability of the model move is defined to be

$$\text{(A.1)} \qquad \alpha(m_1, m_2) = \min\left[1, \lambda(m_1, m_2)\right]$$

where

$$\text{(A.2)} \qquad \lambda(m_1, m_2) = \frac{P(\boldsymbol{\theta}^{m_2}, X^{m_2}, m_2 | D, K) j(m_2, m_1) q_{m_2}(\boldsymbol{u}_{m_2})}{P(\boldsymbol{\theta}^{m_1}, X^{m_1}, m_1 | D, K) j(m_1, m_2) q_{m_1}(\boldsymbol{u}_{m_1})} \times \left| \frac{\partial \Psi}{\partial(\boldsymbol{\theta}^{m_1}, \boldsymbol{u}_{m_1})} \right|$$

In eq. A.2 the first density is the posterior density of the particular model parameters given the data; $j(m_1,m_2)$ is the probability of a move from model m_1 to model m_2; $q_i(\boldsymbol{u}_i)$ is the density of the random variables $\boldsymbol{u}_i$ required to generate the proposed new parameter values in model i. The inclusion of the densities $q_i(\boldsymbol{u}_i)$ ensures that the sampler will show detailed balance — that is to say that for the transition probability between models $P(m,m')$, the Markov chain is reversible: $P(m_1,m_2)\pi(m_1|\circ) = P(m_2,m_1)\pi(m_2|\circ)$, which is a key requirement in such samplers converging on the target distribution. The final term is the change of variables correction where Ψ is the function defining how the parameter vector $\boldsymbol{\theta}^{m_2}$ is generated from $\boldsymbol{\theta}^{m_1}$ and $\boldsymbol{u}_{m_1}$. For the purposes of the survey-based model, the moves between models are defined by either adding or subtracting an age class from the set of separable age classes. Removing an age class, a^s, from the separable set involves an increase in the dimensionality of the system, and we need to generate the new parameters γ^s_{y,a^s}; this is done as follows:

$$\text{(A.3)} \qquad \gamma^s_{y,a^s} = \eta_y + \chi_{a^s} + \boldsymbol{u}_y$$

and $\boldsymbol{u}_y \sim q(\boldsymbol{u})$, where $q(\ldots)$ is the (assumed normal) proposal distribution as defined generically in eq. A.2. When adding a new age class to the separable set, we are decreasing the dimensionality of the system, but we have to generate a new age effect for the newly separable age class a^s, and this is done in a simple deterministic move: $\chi_{a^s} = \chi_{a^s-1}$. This type of move immediately removes the need for the proposal density in the numerator of eq. A.2 and also simplifies the change of variable expression. The final requirement is to define the model move probabilities $j(m_1,m_2)$. The following hierarchy was assumed:

If the maximum separable age a^s is NOT equal to the maximum age or the second smallest age, then a move to a model with maximum separable age class $a^s + 1$ is as equally likely as a move to one with maximum separable age class $a^s - 1$ (for permissible model moves, the model move density ratio in eq. A.2 is unity). The probability of a move that increases or decreases the maximum separable age class by more than 1 is zero. This ensures that moves to models with vastly more or less parameters do not occur and the sampler ascends and descends the space of maximum separable age classes more efficiently. The probability of any feasible move (given the two previous requirements) is equal to 1/20 (so that on average a move is proposed once in every 20 within-model parameter updates).

While work has been done to identify efficient proposal distributions for use in RJMCMC routines (Brooks et al. 2003), these tend to be for systems such as linear hierarchical models where the nested structure makes things a little easier to deal with. A similar nested structure applies here, but it is worth noting that there is some element of trial and error associated with this process. A previous equally plausible scheme was attempted before the one detailed here, which proved inefficient (in terms of the model mixing acceptance rates) relative to this scheme. In terms of "refining" the performance of the sampler, this was done manually whereby the standard deviation of the proposal density $q(\ldots)$ (with a zero mean) was altered until a satisfactory acceptance rate was achieved. This is no different to designing a sampler that enacts the basic Metropolis–Hastings algorithm where there are many possible options — all of which have the desired asymptotic properties — but the speed with which these properties are attained (extracting an independent and identically distributed sample from the distribution of interest) can vary greatly (Gelman et al. 1995).

Following the work detailed in (Brooks and Giudici 1998), convergence of the chains on the full model and parameter posterior was confirmed before performing the model comparison and selection analyses detailed in the main text. The model probabilities used in the Bayes' factor work were approximated using the relative frequency of occurrence of a given model in the Markov chains, once convergence had been verified.

References

Brooks, S.P., and Giudici, P. 1998. Convergence assessment for reversible jump MCMC simulations. Bayesian Statistics 6. Oxford University Press, London, UK.

Brooks, S.P., and Roberts, G.O. 1998. Assessing convergence of Markov chain Monte Carlo algorithms. Stat. Comput. **8**(4): 319-335. doi:10.1023/A:1008820505350.

Brooks, S.P., Giudici, P., and Roberts, G.O. 2003. Efficient construction of reversible jump Markov chain Monte Carlo proposal distributions. J. R. Stat. Soc. B, **65**(1): 3–39. doi:10.1111/1467-9868.03711.

Gelman, A., Carlin, B.J., Stern, H.S., and Rubin, R.B. 1995. Bayesian data analysis. Chapman and Hall, London, UK.

Geman, S., and Geman, D. 1984. Stochastic relaxation, Gibbs distributions, and the Baysian restoration of images. IEEE Trans. Pattern Anal. Mach. Intell. **6**(6): 721–741. doi:10.1109/TPAMI.1984.4767596.

Green, P.J. 1995. Reversible jump Markov chain Monte Carlo computation and Bayesian model determination. Biometrika, **82**(4): 711–732. doi:10.1093/biomet/82.4.711.

Hastings, W.K. 1970. Monte Carlo sampling methods using Markov chains and their applications. Biometrika, **57**(1): 97–109. doi:10.1093/biomet/57.1.97.

Metropolis, N., Rosenbluth, A.W., Rosenbluth, M.N., Teller, A.H., and Teller, E. 1953. Equations of state calculations by fast computing machines. J. Chem. Phys. **21**(6): 1087–1092. doi:10.1063/1.1699114.

Environment trumps predation and spatial factors in structuring cladoceran communities from Boreal Shield lakes

Joshua Kurek, Russ C. Weeber, and John P. Smol

Abstract: Understanding local and regional controls on communities is a major theme in ecology. Are communities mainly determined by local environmental gradients, local biotic interactions, and (or) dispersal processes? To address this, subfossil Cladocera were enumerated from the surface sediments of 50 remote Boreal Shield lakes in northwestern Ontario, Canada, to examine three ecologically relevant explanatory categories. Variation partitioning analyses were used to test and quantify the unique and shared effects of local abiotic measures, local predation factors, and spatial variables as predictors of community composition. Thirty-nine taxa were identified and communities were typically composed of pelagic taxa, such as *Bosmina*, daphniids, and *Holopedium gibberum*. Environmental variables (i.e., pH, lake surface area, specific conductivity, and lake water total aluminum concentration) explained uniquely 8.8% of the variation, which was 2.5 times greater than the unique contributions of predation or dispersal-related spatial variables. Predation and dispersal processes assumed lesser roles in structuring communities. Considering the extensive geographic scale of this study, our paleolimnological approach indicates that broad-scale dispersal limitation of cladocerans seems negligible for the many taxa examined here. Our findings further support the utility of cladocerans as key biological indicators to track environmental changes within Boreal Shield lakes.

Résumé : La compréhension des facteurs locaux et régionaux de contrôle des communautés constitue un des thèmes majeurs de l'écologie. Les communautés sont-elles principalement déterminées par les gradients environnementaux locaux, par les interactions biotiques locales et (ou) par les processus de dispersion? Pour y répondre, nous avons dénombré les cladocères subfossiles des sédiments de surface de 50 lacs éloignés du Bouclier boréal dans le nord-ouest de l'Ontario, Canada, afin d'examiner trois catégories d'explications pertinentes au niveau écologique. Des analyses de partitionnement de la variation ont servi à tester et mesurer les effets uniques et partagés des mesures abiotiques locales, des facteurs locaux de prédation et des variables spatiales comme facteurs prédictifs de la composition de la communauté. Trente-neuf taxons sont reconnus et les communautés se composent typiquement de taxons pélagiques, tels que *Bosmina*, des daphniidés et *Holopedium gibberum*. Les variables du milieu (c'est-à-dire le pH, la superficie du lac, la conductivité spécifique et la concentration totale d'aluminium dans l'eau du lac) expliquent seules 8,8 % de la variation, ce qui est 2,5 fois plus que les contributions uniques de la prédation ou des variables spatiales reliées à la dispersion. La prédation et les processus de dispersion jouent un rôle beaucoup moindre dans la structuration des communautés. Compte tenu de la vaste échelle géographique de notre étude, notre approche paléolimnologique indique que la limitation de la dispersion à grande échelle des cladocères semble négligeable dans le cas de plusieurs des taxons examinés. Nos résultats confirment de plus l'utilité des cladocères comme indicateurs biologiques importants pour suivre les changements environnementaux dans les lacs du Bouclier boréal.

[Traduit par la Rédaction]

Introduction

Ecology provides insight to mechanisms determining community composition across time and space. Several models accounting for variation in species compositions among sites exist and include differing emphases on the controlling factors of community assembly (Leibold et al. 2004). How these models may be tested adequately is actively debated (Legendre et al. 2005; Wagner and Fortin 2005). Of particular interest is whether communities are structured primarily by interactions with their local abiotic environment and (or) other organisms, or are communities a consequence of broad-scale dispersal history? Understanding community composition has relevance to ecological studies employing a "space-for-time" approach, such as in paleolimnology, which often relies on establishing quantitative relationships between

Received 24 January 2011. Accepted 12 May 2011. Published at www.nrcresearchpress.com/cjfas on 10 August 2011.
J2011-0008

Paper handled by Associate Editor Gary W.G. Sprules.

J. Kurek and J.P. Smol. Paleoecological Environmental Assessment and Research Lab (PEARL), Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada.
R.C. Weeber. Canadian Wildlife Service (Ontario region), Environment Canada, 335 River Road, Ottawa, ON K1A 0H3, Canada.

Corresponding author: Joshua Kurek (e-mail: joshua.kurek@gmail.com).

Can. J. Fish. Aquat. Sci. **68**: 1408–1419 (2011) doi:10.1139/F2011-081 Published by NRC Research Press

modern environments and aquatic communities (e.g., benthic invertebrates, chrysophytes, diatoms, zooplankton) to infer past environments from lake sediments (Birks 1998; Smol 2008). To further complicate matters, it is now recognized that different factors structuring communities are also scale-dependant (Cottenie 2005; Telford et al. 2006; Bennett et al. 2010). Because long-term observational data for aquatic ecosystems are exceptionally rare, paleolimnological studies are often the only means to provide context on environmental changes experienced by lake ecosystems (Saros 2009; Smol 2010). However, an assumption rarely addressed is the extent to which assemblages are related to factors other than the main environmental gradient of interest, which is typically an abiotic measure, such as water quality or climate-related measures.

Crustacean zooplankton, such as Cladocera (Crustacea: Branchiopoda), play key roles in the functioning of lake ecosystems because of their intermediate trophic position and interactions with nutrient cycling (Dodson and Frey 2001; Urabe et al. 2002). Consequently, their use as an environmental indicator in paleolimnological studies is now well established (Jeppesen et al. 2001; Korhola and Rautio 2001), largely because of the pioneering research of Frey (1960). Using the remains of cladocerans from lake sediments minimizes seasonal and annual variability and integrates species from multiple habitats compared with surveys of extant Cladocera collected by net hauls through the pelagic zone. Additionally, subfossil and extant Cladocera communities reflect their environment in similar ways (Davidson et al. 2007).

Conflicting evidence of dispersal limitation of cladocerans in acid-sensitive regions, such as the southern Boreal Shield ecozone, is plentiful (reviewed in Gray and Arnott 2009) and highlights the challenge in assessing the relative importance of dispersal controls on the ecological distributions of zooplankton. Larger-bodied freshwater organisms, such as fish, tend to show greater dispersal limitations than smaller aquatic organisms (Beisner et al. 2006; Shurin et al. 2009). As small-bodied organisms with short generation times, cladocerans exhibit relatively high rates of dispersal (Havel and Shurin 2004; Binks et al. 2005; Louette and De Meester 2005). Although evidence of low dispersal rates of zooplankton exists (Bohonak and Jenkins 2003), consensus suggests cladocerans have demonstrated high dispersal ability to respond rapidly to environmental change, a prerequisite as an informative proxy organism in paleolimnological studies. However, this attribute also implies that, in general, cladocerans are effective colonizers and that establishment in "new" environments following dispersal is successful.

Here, we identify key factors structuring Cladocera assemblages among remote lakes of the Boreal Shield ecozone. Specifically, we test the hypothesis that environmental control primarily structures cladoceran communities. We also quantify and test the unique and shared effects of environmental, predation, and dispersal-related spatial variables in explaining cladoceran community composition among our study lakes using variation partitioning methods (Borcard et al. 1992; Legendre and Legendre 1998). We chose these categories of explanatory variables because they represent the current understanding of factors broadly affecting Cladocera, and several are known drivers of zooplankton recovery following improved water chemistry of acidified lakes (Keller and Yan 1998; Gray and Arnott 2009; Valois et al. 2010). Regionally, many Boreal Shield lakes have experienced some chemical recovery from reduced acid precipitation, but currently face other widespread stressors, including climate change, invasive species, and lake water calcium decline. Knowledge of the principal drivers of community assembly is a fundamental issue in ecology and of importance in justifying the use of subfossil organisms as proxies to infer environmental changes. If cladoceran communities are limited by dispersal processes, then the utility of subfossil Cladocera to adequately and rapidly track changing environments may be questioned. Because of the complexity and rapidity of human-induced changes in many aquatic ecosystems, this dilemma (i.e., dispersal limitations or barriers) may be especially acute in studies that provide historical context on the impacts of multiple stressors on aquatic ecosystems (Schindler 2001; Keller 2009; Smol 2010).

Materials and methods

Study lakes and sediment sampling

Study lakes were chosen from a randomized set of 146 remote lakes in northwestern Ontario, Canada, that were identified for sediment core and water chemistry sampling by Environment Canada (EC) (see Kurek et al. 2010*a* for details). Study sites are located near the southern boundary of the Boreal Shield ecozone (Minns et al. 2008). Lakes and surrounding watersheds are underlain by Precambrian Shield bedrock composed of highly metamorphosed volcanic and sedimentary rocks often intruded by massive granitic plutons (Rowe 1972). Regional soils are podzolized and generally shallow and acidic. The area has been altered by erosion and the deposition of fluvial and lacustrine sediments from successive periods of glaciations. As a result, the landscape is varied and consists of rolling hills, rocky outcrops, and lowland peat bogs. The region is also a transition between Boreal and Great Lakes – St. Lawrence forests (Rowe 1972). Common coniferous species include spruce (*Picea glauca* and *Picea mariana*), pine (*Pinus banksiana*, *Pinus strobus*, and *Pinus resinosa*), balsam fir (*Abies balsamea*), tamarack (*Larix laricina*), cedar (*Thuja occidentalis*), and eastern hemlock (*Tsuga canadensis*). Notable broad-leaved species include poplar (*Populus tremuloides* and *Populus balsamifera*), birch (*Betula papyrifera* and *Betula alleghaniensis*), maple (*Acer rubrum* and *Acer saccharum*), and oak (*Quercus rubrum*).

Lakes were sampled as part of a regional water chemistry survey in the autumn of 2008, with EC's aim of estimating acidity critical loads and contributing to air quality policy development. From among the lakes selected for sampling by EC, 50 of the deepest lakes were then chosen for sampling of cladoceran remains from their surface sediments (Fig. 1). Typically, the surveyed lakes were small in surface area (1–151 ha), relatively shallow (5.5–26 m), and slightly acidic to circumneutral (Table 1). Most study lakes were oligotrophic (mean total phosphorus = 8.0 $\mu g \cdot L^{-1}$). Seven lakes in Blocks N and O (also one lake in Block P; Fig. 1) showed relatively high pH (>7.4) and conductivity-related measures (mean specific conductivity = 179 $\mu S \cdot cm^{-1}$), likely because of their location near soils that are relatively well buffered relative to those underlying the other survey lakes. Also nota-

Fig. 1. Map depicting the location of study lakes from northwestern Ontario, Canada. Sites are coded by geographic region (i.e., L, Sioux Lookout; M, Quetico Provincial Park; N, Nakina; O, Hearst; P, Pukaskwa National Park; and Q, Lake Superior Provincial Park).

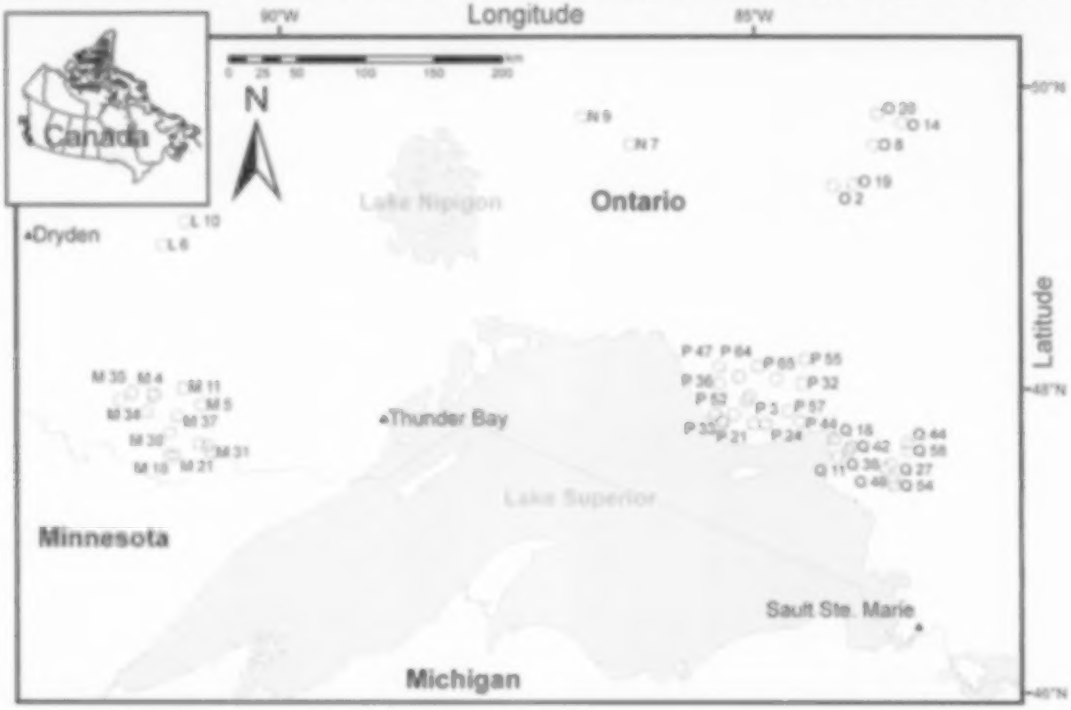

Longitude
90°W
85°W
Latitude
50°N
48°N
46°N
0 25 50 100 150 200 km
N
Canada
Dryden
L 10
L 6
Lake Nipigon
Ontario
N 9
N 7
O 20
O 14
O 8
O 19
O 2
M 35
M 4
M 11
M 5
M 34
M 37
M 30
M 31
M 10
M 21
Thunder Bay
P 47
P 64
P 65
P 55
P 36
P 32
P 52
P 3
P 57
P 33
P 21
P 24
P 44
Q 18
Q 42
Q 44
Q 58
Q 11
Q 38
Q 27
Q 48
Q 54
Lake Superior
Minnesota
Sault Ste. Marie
Michigan

Table 1. Select environmental measures (units in parentheses) from the 49 study lakes.

Variable	Min.	Max.	Mean	SD	Transformation
Surface area (ha)	1.0	151	20.1	32.6	$\log_{10}(x + 1)$
Depth at sampling location (m)	5.5	26	10.6	4.7	Square root
pH ($\log_{10}(1/H^+)$)	4.8	8.1	6.6	0.7	None
Secchi disk depth (m)	0.2	7.6	2.7	1.4	Square root
Specific conductivity ($\mu S \cdot cm^{-1}$)	12.3	380	50.4	68.0	$\log_{10}(x + 1)$
Calcium ($mg \cdot L^{-1}$)	0.7	62.5	7.1	11.2	$\log_{10}(x + 1)$
Dissolved organic carbon ($mg \cdot L^{-1}$)	2.8	21.3	9.9	4.2	Square root
Total phosphorus ($mg \cdot L^{-1}$)	0.002	0.02	0.008	0.004	Square root
Total nitrogen ($mg \cdot L^{-1}$)	0.15	0.82	0.38	0.12	Square root
Total aluminum ($mg \cdot L^{-1}$)	0.004	0.42	0.089	0.087	Square root

Note: SD, standard deviation.

ble, eight lakes located in Blocks M, P, and Q (Fig. 1) showed pH < 6.0. We assume these lakes are naturally acidic owing to their relatively high dissolved organic carbon values (DOC mean = 12.1 $mg \cdot L^{-1}$). The selection of only lakes with water depths ≥5.5 m attempted to control for water depth-related biases between assemblages from ponds and lakes. Additionally, Lake O24 was excluded, despite its water depth, because of its proximity to a major highway (Kurek et al. 2010*a*). Lakes were visited using helicopter and sampled at a midlake position for water chemistry and physical measures (i.e., water depth, Secchi depth, and a thermal profile). A 2 L water sample was collected from the surface waters and divided and processed into subsamples for major ion, nutrient, and metal analyses. Subsamples were then shipped in coolers to the Great Lakes Forestry Centre (GLFC) in Sault Ste. Marie, Ontario. GLFC laboratory participates in quality assurance – quality control programs and uses standard methods for all water chemistry measures. Specific conductivity and pH were measured immediately upon return from the day's fieldwork.

We were not able to use standard gravity coring techniques because of logistical constraints (i.e., a broad-scale helicopter survey designed for water chemistry sampling), but instead used the following approach to collect sediments. Surface sediments were obtained at a midlake position using an Ekman sampler (15 cm × 15 cm × 15 cm) and a 60 mL syringe tube with the basal end removed; hence, this device resembled a tube with a fitted piston. Immediately after retrieval of the Ekman sampler, the syringe tube was pushed slowly through the sediments while the plunger was pulled upward, therefore filling the tube with sediment. The base of the tube was then fitted with a bung. Generally, cores were no greater than 10 cm in length. Cores were stored vertically within a cooler until transported to the laboratory and then sectioned at 1 cm intervals using a modified vertical extruder (Glew 1988). Detailed coring methods are presented in Kurek et al. (2010*a*).

Cladoceran sampling and species data

Cladoceran remains were isolated from lake sediments following standard paleolimnological methods given in Korhola and Rautio (2001). Because of substantial water content, the uppermost 0–1 cm of sediment was examined from each lake to reflect recent lake conditions. Approximately 1 g of wet sediment was deflocculated in warm 5% KOH for ~30 min. The KOH–sediment mixture was then poured onto a 38 μm mesh sieve and rinsed with running water. Sediment retained on the sieve was then transferred with distilled water into a small vial, and several drops of ethanol and safranin–glycerin solution were added. Next, 50 μL of the solution was pipetted onto each slide, and after evaporation, another 50 μL was added and allowed to evaporate. Permanent slides were made using glycerine jelly and cover slips.

Specimens were identified using bright-field illumination at 200× or 400× magnification. Identifications followed the subfossil taxonomy of Szeroczyńska and Sarmaja-Korjonen (2007), with limited reference to Smirnov (1974) and Frey (1982). Bosminid headshields were assigned to the genus level because of the challenge in consistently locating the lateral headpore. Daphniidae were separated into four groups based on the middle pectin of the postabdominal claws: the *Daphnia pulex* group (*Daphnia pulicaria*, *D. pulex*), the *Daphnia catawba* group (*D. catawba*, *Daphnia minnehaha*), the *Daphnia longispina* group (*Daphnia ambigua*, *Daphnia dentifera*, *Daphnia dubia*, *Daphnia longiremis*, *Daphnia mendotae*), and *Ceriodaphnia*. Each cover slip was entirely scanned and all remains (i.e., identifiable body parts including carapaces, headshields, postabdomens, postabdominal claws, exopodite segments, and caudal furca) were tabulated. Because of the disarticulation and fragmentation of cladoceran subfossils, the most abundant remain of each taxon was used to calculate the number of individuals at each study site (Frey 1986; Korhola and Rautio 2001). Any taxon with remains summing to half an individual were rounded up to one individual. A minimum counting effort of between 70 and 100 individuals was targeted as a reliable estimate of the subfossil cladoceran assemblage (Kurek et al. 2010*b*). A total of 49 out of 50 lakes met this criterion (number of individuals: min. = 114, max. = 283, mean = 168). Cladoceran data are expressed as a percent relative abundance of the total number of individuals counted for each site. The relative abundances of only the common cladocerans (i.e., >2% in at least two lakes) are presented (Fig. 2 and Table 2).

Explanatory variables: environmental, predation, and spatial data sets

For each study lake, 24 environmental variables were measured and considered as potentially important predictors of cladoceran communities. The complete environmental data set included pH, specific conductivity (COND), alkalinity (ALK), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), sulfate (SO_4), chloride (Cl), dissolved silica (SiO_2), nitrate (NO_3), ammonium (NH_4), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total phosphorus unfiltered (TP), soluble reactive phosphorus (SRP), total nitrogen (TN), total aluminum (Al), iron (Fe), manganese (Mn), zinc (Zn), maximum water depth (DEPTH) at the sampling location, Secchi disk depth (SECCHI), and lake surface area (AREA). At Lake N9, the Secchi disk was visible at the sediment–water interface; therefore, SECCHI was assigned the value of DEPTH. At Lake M10, no Secchi disk depth was recorded; therefore, SECCHI was estimated using DOC and linear regression ($y = -0.23x + 4.95$, $P \leq 0.0001$). Three variables (i.e., Cl, NO_3, and Mn) were below the level of detection in many lakes and were therefore eliminated. Most environmental variables were transformed using either a square-root or $\log_{10}(x + 1)$ transformation to improve normality and homogeneity of variances (Table 1). To reduce correlations among environmental predictors, we chose to retain only COND from a highly, positively correlated (all $r > 0.96$) group of related water chemistry variables (i.e., COND, ALK, Ca, Mg, and DIC). In addition, to further minimize the influence of correlated variables, we ran partial redundancy analyses (pRDAs) constrained to each variable alone. Variables were retained for further consideration only if they explained a significant ($P \leq 0.05$) amount of variation in pRDAs. Although Zn was initially identified as a significant predictor, several lakes had high Zn values. When these sites were removed from the pRDA, Zn was no longer a significant predictor. Therefore, we chose to eliminate Zn as an explanatory variable rather than remove those sites with high square-root-transformed Zn values. Lastly, manual forward selection, beginning with pH, was used to identify a minimum subset of environmental variables, each of which explained a significant ($P \leq 0.05$) amount of variation in the cladoceran data. Although Al explained the most variation and ~0.9% more variation than pH, pH was selected first for ecological and heuristic reasons. To assess the robustness of each significant environmental variable, four additional pRDAs were run with all the remaining significant variables as covariables.

The predation data set is based on mandible counts of the larvae of three species and one subgenus of *Chaoborus* (Diptera: Chaoboridae): *Chaoborus americanus*, *Chaoborus trivittatus*, *Chaoborus flavicans*, and *C.* (subgenus *Sayomyia*). *Chaoborus* (*Sayomyia*) includes both *Chaoborus albatus* and *Chaoborus punctipennis*, as these two species cannot be distinguished using only mandible remains (Uutala 1990). Principal components analysis (PCA) of transformed *Chaoborus* data (covariance matrix) was used to summarize the *Chaoborus* communities and derive a gradient of predation pressure on cladoceran assemblages among our 49 study lakes (Fig. 3). Chaoborids are gape-limited ambush predators that prey on a variety of zooplankton, including cladocerans, and are known to affect the composition and abundance of zooplankton communities (Moore et al. 1994; Riessen 1999; Keller et al. 2002). Additionally, *C. americanus* is indicative of habitats with low planktivorous fish predation or even fishless lakes (Sweetman and Smol 2006; Garcia and Mittelbach 2008; Kurek et al. 2010*a*). Selective predation has also been shown to be important in structuring the body size of zooplankton communities (Brooks and Dodson 1965). There-

Fig. 2. Relative abundances of common cladocerans (i.e., >2% abundance in at least two lakes) from the 49 study lakes. Lakes are ordered according to lake water pH.

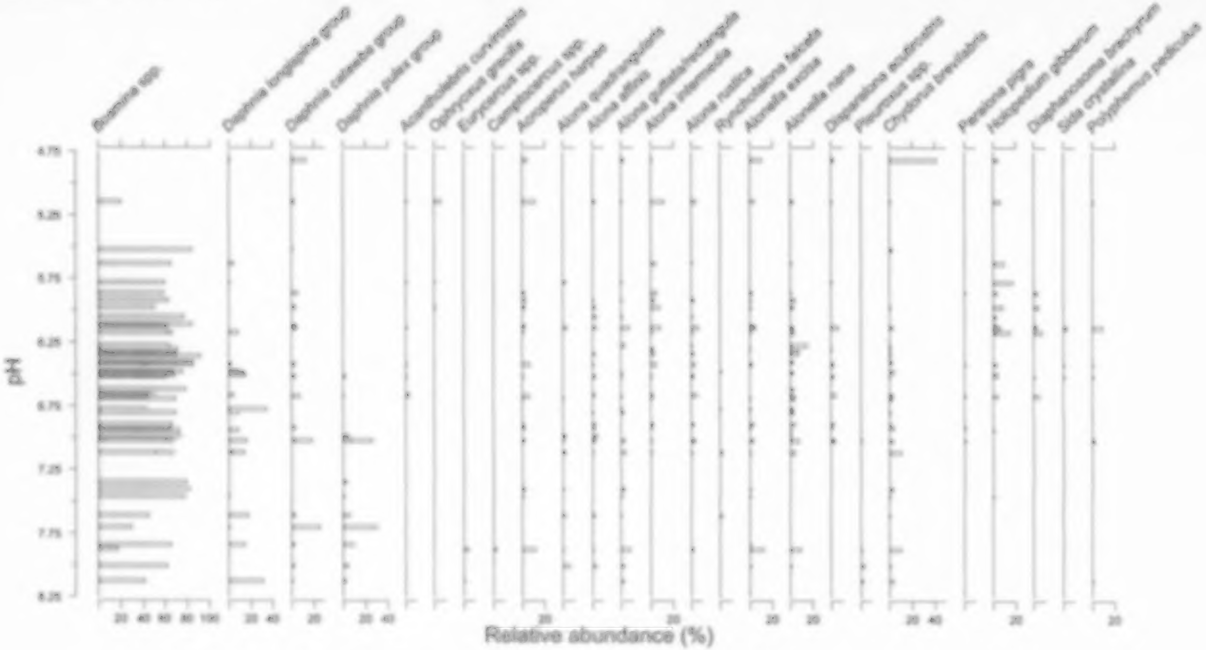


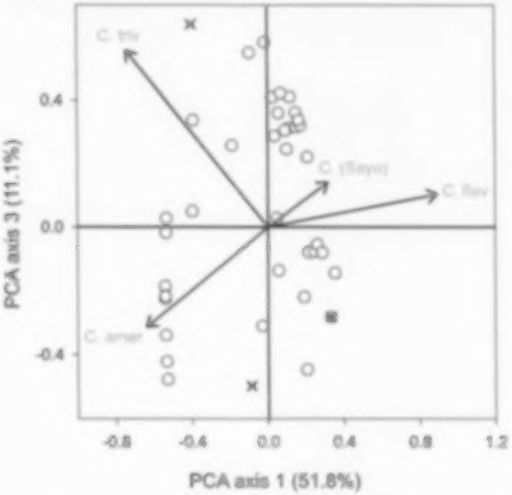
Table 2. Common cladocerans (i.e., >2% relative abundance in at least two lakes) from the 49 study lakes.

Taxon	Code	Occurrence (% of lakes)	Max. (%)	Mean (%)	Hill's N2
Acantholeberis curvirostris O.F. Müller	Acur	44.8	4.1	0.59	14.3
Acroperus harpae Baird	Ahar	91.8	12.2	2.1	18.7
Alona affinis Leydig	Aaff	87.8	5.0	1.5	28.2
*Alona guttata/rectangula** Sars	Agut	81.6	8.7	1.7	23.0
Alona intermedia Sars	Aint	75.5	12.8	2.0	18.6
Alona quadrangularis O.F. Müller	Aqua	61.2	6.5	0.98	18.5
Alona rustica Scott	Arus	79.5	7.8	1.6	23.0
Alonella excisa Fischer	Aexc	85.7	13.9	2.0	18.4
Alonella nana Baird	Anan	83.6	15.2	2.7	21.8
*Bosmina** Baird	Bosm	97.9	92.2	60.4	43.4
*Camptocercus** Baird	Camp	14.2	3.4	0.15	3.6
Chydorus brevilabris Frey	Cbre	97.9	42.2	3.2	10.8
*Daphnia catawba group**	Dcat	57.1	26.7	2.7	11.0
*Daphnia longispina group**	Dlon	65.3	35.6	4.9	13.2
*Daphnia pulex group**	Dpul	32.6	32.0	2.4	6.4
Diaphanosoma brachyurum Liévin	Dbra	42.8	8.1	0.91	11.3
Disparalona acutirostris Birge	Dacu	67.3	7.0	1.1	20.1
*Eurycercus** Baird	Eury	36.7	5.2	0.39	8.7
Holopedium gibberum Zaddach	Hgib	61.2	17.8	2.4	14.0
Ophryoxus gracilis Sars	Ogra	53.0	6.7	0.56	11.3
Paralona pigra Sars	Ppig	61.2	2.7	0.68	22.39
*Pleuroxus** Baird	Pleu	34.6	4.0	0.42	10.1
Polyphemus pediculus Linné	Pped	61.2	10.2	0.87	12.3
Rynchotalona falcata Sars	Rfal	18.3	2.9	0.26	6.2
Sida crystallina O.F. Müller	Scry	63.2	4.7	0.60	18.3

Note: Code corresponds to taxa abbreviations from Fig. 3*b*. Occurrence is a percentage of the study lakes. Hill's N2 is a diversity index that weights abundance by occurrence.

*Denotes grouping of possibly more than one species.

Fig. 3. Biplot of axes 1 and 3 from a principal components analysis (PCA) of *Chaoborus* mandibles from 49 study lakes. Lakes with mandible counts of fewer than five are coded as crosses. Taxa are abbreviated and include *C. americanus*, *C. trivittatus*, *C. flavicans*, and *C.* (*Sayomyia*).



fore, we interpret the gradient of predation estimated by PCA as an indirect measure of the influence of both *Chaoborus* and planktivorous fish on cladoceran assemblages. *Chaoborus* mandibles were isolated from the uppermost 0–2 cm of sediment. Detailed sampling methods and results of the 146 lake *Chaoborus* survey are presented elsewhere (Kurek et al. 2010*a*). Seven lakes yielded fewer than five mandibles, although they are still included (number of mandibles: min. = 0, max. = 202, mean = 30), despite the minimum counting guidelines for estimating *Chaoborus* community structure from subfossil mandibles (Quinlan and Smol 2010). Prior to PCA, *Chaoborus* mandible counts were transformed using the Hellinger transformation (Legendre and Gallagher 2001). We then used the forward selection procedure outlined in Blanchet et al. (2008) to select a minimum subset of explanatory variables (i.e., PCA sample scores) from the *Chaoborus* PCA axes. This procedure corrects for an overestimation of explained variation and combines selection based on a preselected significance level ($P \leq 0.05$) and the adjusted coefficient of determination (R^2_{adj}) of the global model (Peres-Neto et al. 2006).

A data set of spatially derived variables was generated from our study lakes to examine the influence of spatial patterns as significant predictors of cladoceran assemblages. Specifically, principal coordinates of neighbour matrices (PCNM) was applied (Borcard and Legendre 2002; Dray et al. 2006). PCNM is a widely used and effective technique to model complex, nonlinear spatial patterns. Basically, PCNM is a numerical technique that generates a set of spatial explanatory variables that have structure at scales defined by a matrix of geographic position among sites. PCNM eigenvectors used in constrained ordination often explain more variation in community data than variables generated by polynomial trend-surface analysis (Borcard et al. 2004; Legendre et al. 2005; Dray et al. 2006), which can be limited by a coarse representation of space, strong correlations among terms, and irregular sampling design. First, we converted longitude and latitude into centered *x*, *y* coordinates in units of kilometres. Next, PCNM was applied, with Euclidean representation and a truncation distance of 100. This yielded 20 PCNM eigenvectors. We then selected a minimum subset of spatial variables following the procedure of Blanchet et al. (2008).

Direct gradient analyses and variation partitioning

Prior to analyses, cladoceran data were Hellinger-transformed to better utilize Euclidean-based ordination methods (Legendre and Gallagher 2001). This transformation reduces the weight of the abundant species. To test the influence of environmental (ENV), predation (PRED), and spatial (SPACE) variables as predictors of cladocerans among our 49 study lakes, direct gradient analyses were used. Detrended correspondence analysis showed that gradient lengths of the axes were low (<2 standard deviation units); therefore, linear-based ordinations (redundancy analysis, RDA) were performed (Birks 1998).

Only the reduced subsets of forwarded-selected ENV, PRED, and SPACE variables were used in the final RDA (Fig. 4). The significance of the first two canonical axes was tested to determine if these axes effectively represented cladoceran variation with respect to the explanatory variables (Table 3). Canonical coefficients and interset correlations of the variables with the axes were also examined (Table 3). Variation partitioning of RDA models, including pRDAs, were used to quantify and provide insight on community variation explained by each data set. For example, a significant ENV fraction of variation, after controlling for PRED and SPACE, may indicate the importance of local environmental factors as a key control on cladocerans. Our partitioning methods follow those given in Borcard et al. (1992), except they were adapted for three explanatory data sets (Legendre et al. 2005). The variation captured by each of the RDA models allows us to quantify fractions of variation in the community data that are uniquely explained by ENV, PRED, or SPACE and also shared variation between data sets, in addition to unexplained variation (Legendre and Legendre 1998). Variation explained was adjusted for multiple comparisons (R^2_{adj}), as per recommendations of Peres-Neto et al. (2006), because of the biases in interpreting unadjusted values.

Numerical analyses

All analyses were completed in R version 2.11.1 (R Development Core Team 2010) using the following three packages: (1) vegan (Oksanen et al. 2010) for PCA, RDA, variation partitioning, associated permutation tests, and PCNM; (2) SoDA (Chambers 2008) for generating centered geographic coordinates; and (3) packfor (Dray et al. 2009) to apply the forward selection procedures of Blanchet et al. (2008).

Results

Cladoceran relative abundances

A total of 39 Cladocera taxa were identified from subfossil remains in the surface sediments of our study lakes. Twenty-

Fig. 4. Redundancy analysis (RDA) showing the (*a*) site scores and (*b*) species scores from 49 study lakes, plotted relative to the (*c*) significant variables. Only common cladocerans are shown. Note different scaling of axes. Sites are coded by region (i.e., Sioux Lookout, open triangles; Quetico Provincial Park, gray circles; Nakina, solid squares; Hearst, gray triangles; Pukaskwa National Park, open circles; and Lake Superior Provincial Park, solid circles).

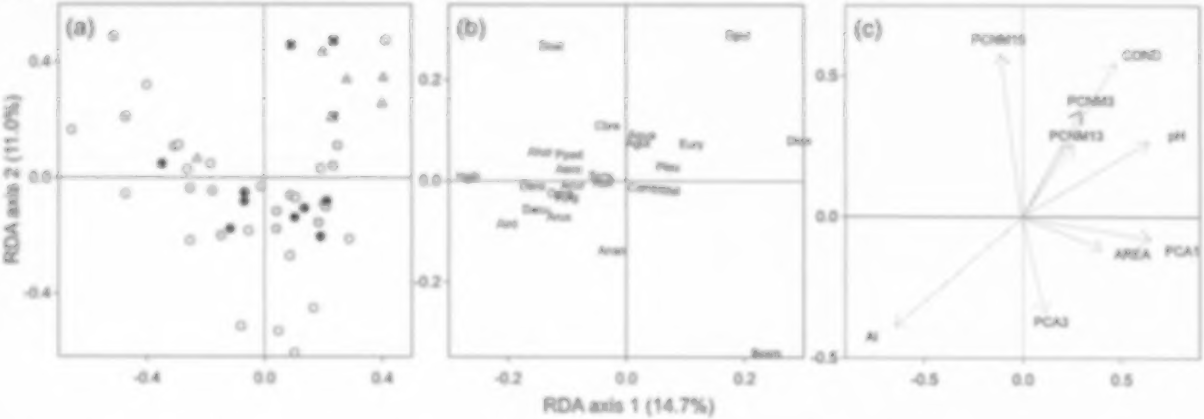


Table 3. RDA summary including canonical coefficients and interset correlations of variables with the first two axes.

	Axis 1	Axis 2
Eigenvalues	0.032	0.024
Significance test of axis *F* ratio	9.89*	7.43*
Canonical coefficients		
pH	0.08	−0.21
AREA	0.06	0.07
COND	−0.07	0.57
Al	−0.30	−0.40
PCA 1	0.20	−0.23
PCA 3	0.05	−0.03
PCNM 15	−0.06	0.40
PCNM 3	0.09	0.00
PCNM 13	0.00	0.07
Interset correlations		
pH	0.66	0.20
AREA	0.47	−0.09
COND	0.51	0.40
Al	−0.64	−0.30
PCA 1	0.71	−0.08
PCA 3	0.12	−0.26
PCNM 15	−0.10	0.44
PCNM 3	0.29	0.29
PCNM 13	0.23	0.20

*Significance level at $P \leq 0.05$.

five taxa occurred in at least two lakes at >2% abundance and were therefore considered common (Fig. 2 and Table 2). The pelagic taxon *Bosmina* spp. was especially widespread and showed high abundances of >40% in 43 of 49 lakes. *Chydorus brevilabris*, a littoral species, was the second most common taxon and occurred at 48 of 49 lakes with a mean abundance of 3.2%. Among the daphniids, in general, the *D. longispina* group was more widespread and abundant than either the *D. catawba* or *D. pulex* groups. Typically, the *D. pulex* group occurred in greater abundances in lakes with pH > 6.5, whereas *Holopedium gibberum* occurred at higher abundances in lakes with pH < 6.5. Although *Eurycercus* spp. rarely occurred at >2% abundance, this taxon was more common in lakes with higher pH. Generally, *Paralona pigra* and *Diaphanosoma brachyrum* were distributed widely among lakes with a pH of <7. Taxa of the genera *Alona* and *Alonella* were distributed broadly among most of our study lakes.

Forward selection of explanatory variables from each of the three data sets

Forward selection procedures using RDA recognized four environmental variables that each explained a significant portion of the variation in the cladoceran data. In order of explanatory power, these were pH, AREA, COND, and Al. Variance inflation factors of the environmental variables were all <6.2. In pRDAs, with the three other significant variables as covariables, each environmental variable retained its explanatory significance (not shown). PCA axis 1 and PCA axis 3 were the only significant predictor variables within the predation data set. Cumulatively, these PCA axes explained 11.4% of the variation in the cladoceran data (Table 4). Of the 20 PCNM spatial variables considered, three were identified as significant in forward selection procedures and included PCNMs 3, 13, and 15. Together, these spatial variables explained 9.4% of the variation in the cladoceran data (Table 4).

Community variation explained by RDA and variation partitioning

A total of nine significant variables were identified by forward selection procedures and used in the variation partitioning analysis (Table 4). ENV, PRED, and SPACE data sets each explained significant ($P \leq 0.05$) proportions of variation in the relative abundances and distributions of cladoceran assemblages among the 49 study lakes. Combined, all three data sets explained 28.7% of the variation in the cladoceran data (Table 4). All variance inflation factors were less than 3, except for the inflation factors of pH and COND, which

Table 4. Variation partitioning of cladoceran community data using RDA and pRDA.

Explanatory data	Covariable(s)	R^2_{adj}	DF	DF_{res}	*F*	*P*
ENV	None	20.1 (26.8)	4	44	4.03	0.001
PRED	None	11.4 (15.0)	2	46	4.08	0.001
SPACE	None	9.4 (15.1)	3	45	2.67	0.001
ENV+PRED	None	25.3 (34.7)	6	42	3.72	0.001
ENV+SPACE	None	25.2 (36.1)	7	41	3.31	0.001
PRED+SPACE	None	19.8 (28.2)	5	43	3.38	0.001
ALL three data sets	None	28.7 (42.0)	9	39	3.14	0.001
ENV	PRED+SPACE	8.8	4	39	2.32	0.001
PRED	ENV+SPACE	3.4	2	39	1.88	0.020
SPACE	ENV+PRED	3.3	3	39	1.65	0.040
ENV	SPACE	15.7	4	41	3.37	0.001
ENV	PRED	13.9	4	42	3.15	0.001
PRED	SPACE	10.4	2	43	3.93	0.001
PRED	ENV	5.2	2	42	2.54	0.003
SPACE	ENV	5.0	3	41	1.99	0.010
SPACE	PRED	8.4	3	43	2.62	0.001

Note: The three explanatory data sets were environmental (ENV), predation (PRED), and spatial (SPACE). Adjusted variation explained (R^2_{adj}) is based on the recommendations of Peres-Neto et al. (2006). Uncorrected values are reported within brackets. Contributions of variation were tested by 999 permutations under the reduced model. When calculating the *F* distribution, DF is the numerator and DF_{res} is the denominator. Unexplained variation equals 71.3%.

were 7.0 and 8.0, respectively. The greatest amount of variation explained by one data set was by ENV (20.1%) and indicated a strong environmental influence of primarily water chemistry variables. Nonetheless, shared variation between data sets was also modest and ranged between 6.9% (ENV and PRED) and 1.7% (PRED and SPACE). The spatially structured environmental effect was 4.4%. ENV explained (uniquely) 8.8% of the variation, which was approximately 2.5 times greater than the unique contributions of PRED or SPACE, after shared variation was accounted for by pRDAs (Table 4). The majority of the variation in the cladoceran data was unexplained (71.3%) by the forward-selected ENV, PRED, and SPACE variables considered in this analysis.

Based on interset correlations, pH, AREA, COND, and PCA 1 were all positively correlated with RDA axis 1, whereas Al was negatively correlated with RDA axis 1 (Table 3). The strongest interset correlations were associated with pH, Al, and PCA 1 gradients (0.66, –0.64, and 0.71, respectively). COND and PCNM 15 showed positive, although modest, correlations with RDA axis 2. PCA 3, PCNM 3, and PCNM 13 all showed low correlations (less than 0.30) with the first two RDA axes. In general, a biplot of the RDA site scores showed a distinction between assemblages collected across the gradients (Fig. 4*a*). Sites within the right quadrants showed relatively high pH, AREA, and COND values, whereas sites within the left quadrants showed high Al values and relatively lower values of pH, AREA, and COND. Also, sites within the right quadrants were associated with increased values of PCA 1, whereas sites within the left quadrants were associated with decreased values of PCA 1. PCA 1 is interpreted as primarily a predation gradient of *Chaoborus* assemblages dominated by *C.* (*Sayomyia*)/*C. flavicans* (higher PCA 1 values) or *C. americanus*/*C. trivittatus* (lower PCA 1 values) (Fig. 3). PCA 3 is generally characterized by increased *C. americanus* (lower PCA 3 values), and greater abundances of the three other taxa were typically associated with higher PCA 3 values.

RDA species scores indicate correlations with the constraining variables (Fig. 4*b*). Generally, the *D. pulex* group, the *D. longispina* group, and *Eurycercus* spp. were positively related to constraining variables that increased in value within the upper-right quadrant (i.e., pH and COND). In the left quadrants, *H. gibberum*, *Alona intermedia*, *Alona rustica*, *Alonella nana*, and *Disparalona acutirostris* were negatively correlated with these variables and positively associated with Al. Typically, *Bosmina* spp. increased in abundance as AREA, PCA 1, and PCA 3 increased, whereas the *D. catawba* group, *Acroperus harpae*, and *C. brevilabris* were negatively related to AREA, PCA 1, and PCA 3. Several taxa plotted near the origin and in general indicated minimal correlation with the constraining variables (Fig. 2).

Discussion

Our study examines the shared and unique effects of environmental, predation, and dispersal-related explanatory categories on community composition of cladoceran zooplankton. The key finding is that local environmental factors (i.e., pH, lake surface area, specific conductivity, lake water total aluminum concentration) contributed most, compared with predation and spatial factors, in explaining variation in cladoceran assemblages at the broad geographic scale (~1.8 × 10^5 km^2) of the remote lakes surveyed. Overall, these results further support the utility of subfossil cladocerans as indicators with great potential to track environmental changes from lakes in the Boreal Shield ecozone. Although statistical methods showed that all three explanatory categories contributed significantly to describing community variation, local environmental factors explained about 2.5 times more unique variation than predation or spatial variables. Environmental control by primarily acidity-related variables appears to be the dominant structuring force. Valois et al. (2010) also demonstrated strong environmental control when examining extant zooplankton assemblages and partitioning variation by abiotic and biotic explanatory factors in lakes recovering from acidification near Sudbury, Ontario. Our results do not imply that predation or broad-scale dispersal processes are trivial, but that they are not the principal drivers of cladoceran community composition.

There is an exceptionally rich body of literature from the Boreal Shield ecozone highlighting the importance of acidity-related variables on cladoceran community composition and physiological responses (e.g., Keller and Yan 1998; Walseng et al. 2003; Gray and Arnott 2009). Overall, the study lakes were characterized by pelagic taxa such as *Bosmina* spp., daphniids, and *H. gibberum*, which make up on average 70% of the assemblage. Lake acidity-related variables and ion concentrations were the major environmental gradients influencing the subfossil communities (DeSellas et al. 2008). In general, acid lakes ($\text{pH} < 6.0$) showed reductions in daphniid occurrence and to some extent abundance (Keller and Pitblado 1984). The *D. pulex* group showed an affinity for lakes with higher pH, whereas the other daphniid groups were more broadly distributed. Similar to other regional surveys, we also recognized a strong negative association between *H. gibberum* and daphniid relative abundances (Keller and Pitblado 1984; Yan et al. 1988; Valois et al. 2010). Hessen et al. (1995) suggested that *Holopedium* has a competitive advantage over *Daphnia* at low pH and decreased calcium concentrations, while Valois et al. (2010) indicated that greater metal tolerance of *H. gibberum* may be important to its competitive advantage. While our approach is mainly exploratory, the patterns demonstrate that subfossil cladocerans are largely indicative of water quality, particularly acidity-related measures that are often strongly correlated, such as pH, specific conductivity, and lake water total aluminum concentration.

At extensive geographic scales ($\sim 1 \times 10^4$ to $1 \times 10^6\ \text{km}^2$), typical of many lake surveys relating subfossil community composition to explanatory variables, environmental factors trump the influence of spatial factors (Bennett et al. 2010). Surprisingly, this pattern exists despite the widely different trophic levels, body size, and life-history strategies of the subfossil communities examined to date (i.e., diatom and cladoceran). Shurin et al. (2009) demonstrated that small-bodied freshwater taxa, ranging in size from unicellular plankton to macroinvertebrates, are less constrained by dispersal limitation than larger taxa. However, spatial factors, representing a coarse measure of dispersal processes of subfossil communities, may be more relevant than environmental heterogeneity as geographic scale expands (Cottenie 2005). Pinel-Alloul et al. (1995) identified water chemistry gradients and biotic factors (bottom-up and top-down factors) as the main processes structuring living zooplankton communities from 54 Quebec lakes; spatial factors were not significant predictors of community variability. Of the few comparable subfossil cladoceran investigations, conflicting evidence of the importance of environmental versus spatial variables exists. For example, Bos and Cumming (2003) reported greater independent environmental (18%) than spatial (8%) explanatory power, whereas Sweetman et al. (2010) showed greater unique effects of spatial (13.6%) than environmental (7.9%) variables. It is challenging to reconcile these findings given each author's differing approach of estimating spatial factors. Similarly, studies of living Cladocera and other zooplankton also emphasize that local environmental conditions and dispersal processes, to some degree, both influence community variation (Shurin 2000; Cottenie et al. 2003; Gray and Arnott 2011), although as the size of an organism increases and motility decreases, dispersal processes may become more influential in shaping their contribution to aquatic communities (Beisner et al. 2006). The critical body size where dispersal limitation increases occurs between that of invertebrates and vertebrates (Shurin et al. 2009). Declerck et al. (2010) showed that in wetland communities of living Cladocera, environmental control occurs at the smallest scale (within wetlands), whereas spatial factors become more important at the largest scale (among valleys). In a meta-analysis, ~75% of communities showed either a significant unique environmental component or significant unique components of both environmental and spatial factors, leading to the overall conclusion that environmental heterogeneity and species-sorting dynamics were dominant (Cottenie 2005). Clearly, additional study of the role of multiple spatial scales and its influence on the structuring factors of subfossil communities is warranted.

A unique feature of our paleolimnological study is the inclusion and subsequent significance of predation as an explanatory factor. Rarely is predation considered as a structuring agent of subfossil communities, much less quantitatively assessed using direct ordination analyses; although, there exists some debate as to the consequences of bottom-up or top-down controls on zooplankton communities in paleolimnological studies (Leavitt et al. 1989; Davidson et al. 2007; Korosi et al. 2008). Here, we show the importance of predation as an important structuring factor of cladoceran assemblages among our 49 study lakes. Our predation gradient likely represents the effects of both gape-limited invertebrate predation and, to some extent, predation from planktivorous fish (i.e., in lakes without the fishless indicator *C. americanus*). These predation regimes may have opposing effects on the size structure of zooplankton (Brooks and Dodson 1965). PCA 1 and PCA 3 of the *Chaoborus* data set explained (uniquely) 3.4% of variation in the cladoceran data, approximately 2.5 times less than environmental factors. Similarly, Valois et al. (2010) also noted lower relative importance of predation (5%) compared with abiotic (19%) variables in explaining zooplankton community patterns in previously acidified lakes.

Larger chaoborids, such as *C. americanus* (negatively correlated with PCA 1 and PCA 3), are sensitive to planktivorous fish predation and consequently occur in greater abundances in lakes <10 ha and in lakes with low pH and high lake water total Al concentrations (Kurek et al. 2010*a*). Smaller chaoborids, such as *C. flavicans* (positively correlated with PCA 1 and PCA 3), are more common in lakes higher in pH and conductivity-related variables (Kurek et al. 2010*a*). Within this data set, *C. americanus* and *C. trivittatus* are negatively correlated with *Bosmina* spp., the *D. pulex* group, and the *D. longispina* group; whereas, *C.* (*Sayomyia*) and *C. flavicans* are positively associated with those three common cladoceran taxa. The *Chaoborus* spp. predation gradient as a significant explanatory factor is contrary to findings from other subfossil cladoceran studies in the Boreal Shield ecozone (DeSellas et al. 2008; Korosi et al. 2008). This may be the result of how predation was measured in each study (i.e., PCA of subfossil chaoborid mandible counts versus density of living larvae) or the greater number of lakes in our study with high abundances of *C. americanus* and estimated fishless conditions or low planktivorous fish predation pressure (Kurek et al. 2010*a*). Understanding predation and other top-down controls of subfossil aquatic communities is a research avenue needing additional exploration given the widespread occurrences of biological invasions in Canadian fresh waters (Schindler 2001).

Several limitations of our study should be considered, as unexplained variation was 71%. Because this multivariate analysis is correlative and tests the importance of three explanatory categories, much depends on the variables included in each category. If relevant variables or interactions were not included (e.g., temperature, light regime, absolute abundances of key predators), or if one category was more poorly represented than others, the relative importance of each category may not be properly assessed. This limitation is likely to bias the predation category. Additionally, our approach assumes that instantaneous conditions (e.g., water chemistry values) influence community patterns more than seasonal variability and that variation within lakes is minimal compared with the variation between lakes. However, relative to studies of extant communities, our paleolimnological approach likely reduces these sources of variability. Uncertainty in subfossil taxonomy leads to lumping of species into higher taxonomic levels, for example, the bosminid and daphniid groups. This likely results in a loss of ecological information and may be problematic when examining spatial patterns. Diatom species grouped at the genus level indicate stronger environmental control, although Bennett et al. (2010) suggest that any biogeographic patterns present at lower taxonomic levels may ultimately be lost. Taxonomic uncertainty will always be part of any paleolimnological study using subfossil remains. Also, the interpretation of spatial variables needs mention. Spatial factors are often interpreted as dispersal-related variables or as unmeasured environmental factors structured by spatial processes (Legendre and Legendre 1998). This is a confounding issue and remains a challenge to address. Because we have included ecologically relevant explanatory variables in the environmental and predation data sets, we consider the spatial factors as a surrogate of dispersal limitations in taxa. These limitations are not unique to our study, as they occur in most investigations based upon multivariate analyses of community data.

To conclude, reliable environmental inferences based on multivariate community data require, at a minimum, a basic understanding of key abiotic and biotic factors influencing communities. In this examination of cladoceran community variability from 49 remote Boreal Shield lakes, we demonstrate that community composition primarily is driven by acidity-related environmental factors that include pH, lake surface area, conductivity, and lake water total aluminum concentration. Secondarily, predation and dispersal-related spatial factors explain significant unique components of cladoceran variation, although much less than local environmental factors. Additionally, the relevance of local and regional factors structuring zooplankton communities is likely scale-dependant. Overall, this suggests that at the broad geographic scale of this study, local environmental control is the dominant structuring factor of cladoceran communities, and dispersal limitation is much less important. Furthermore, our findings provide insight to the potential predictive power of crustacean zooplankton to adequately track environmental changes in a paleolimnological approach. Given that multiple anthropogenic stressors will continue to have a widespread effect on lake ecosystems throughout the Boreal Shield ecozone, the ecological information afforded by subfossil cladoceran studies has potential to contribute to a greater understanding of environmental changes within lake ecosystems.

Acknowledgements

The water chemistry analyses and sediment core collection was supported by Environment Canada's Acid Rain Program. Fieldwork was performed by R. Semkin, D. Gilroy, T. Mamone, R. Neuruether, and N. Stewart. Additional funding was provided by Natural Sciences and Engineering Research Council of Canada (NSERC) grants to JPS and an NSERC Postdoctoral Fellowship to JK. We also thank J. Glew and C. Grooms for technical assistance with sediment subsampling. Comments by S.E. Arnott, B.F. Cumming, J.B. Korosi, and R. Semkin improved early drafts of this manuscript. Two anonymous reviewers and an Associate Editor also provided constructive comments.

References

Bennett, J.R., Cumming, B.F., Ginn, B.K., and Smol, J.P. 2010. Broad-scale environmental response and niche conservatism in lacustrine diatom communities. Glob. Ecol. Biogeogr. **19**: 724–732.

Beisner, B.E., Peres-Neto, P.R., Lindstrom, E.S., Barnett, A., and Longhi, M.L. 2006. The role of environmental and spatial processes in structuring lake communities from bacteria to fish. Ecology, **87**(12): 2985–2991 doi:10.1890/0012-9658(2006)87[2985:TROEAS]2.0.CO;2. PMID:17249222.

Binks, J.A., Arnott, S.E., and Sprules, W.G. 2005. Local factors and colonist dispersal influence crustacean zooplankton recovery from cultural acidification. Ecol. Appl. **15**(6): 2025–2036. doi:10.1890/04-1726.

Birks, H.J.B. 1998. Numerical tools in palaeolimnology — progress, potentialities, and problems. J. Paleolimnol. **20**(4): 307–332. doi:10.1023/A:1008038808690.

Blanchet, F.G., Legendre, P., and Borcard, D. 2008. Forward selection of explanatory variables. Ecology, **89**(9): 2623–2632. doi:10.1890/07-0986.1. PMID:18831183.

Bohonak, A.J., and Jenkins, D.G. 2003. Ecological and evolutionary significance of dispersal by freshwater invertebrates. Ecol. Lett. **6**(8): 783–796. doi:10.1046/j.1461-0248.2003.00486.x.

Borcard, D., Legendre, P., Avois-Jacquet, C., and Tuomisto, H. 2004. Dissecting the spatial structure of ecological data at multiple scales. Ecology, **85**(7): 1826–1832. doi:10.1890/03-3111.

Borcard, D., and Legendre, P. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecol. Model. **153**(1–2): 51–68. doi:10.1016/S0304-3800(01)00501-4.

Borcard, D., Legendre, P., and Drapeau, P. 1992. Partialling out the spatial component of ecological variation. Ecology, **73**(3): 1045–1055. doi:10.2307/1940179.

Bos, D.G., and Cumming, B.F. 2003. Sedimentary cladoceran remains and their relationship to nutrients and other limnological variables in 53 lakes from British Columbia, Canada. Can. J. Fish. Aquat. Sci. **60**(10): 1177–1189. doi:10.1139/f03-097.

Brooks, J.L., and Dodson, S.I. 1965. Predation, body size, and composition of plankton. Science, **150**(3692): 28–35. doi:10.1126/science.150.3692.28. PMID:17829740.

Chambers, J.M. 2008. SoDA: functions and examples for "Software for Data Analysis". R package version 1.0-3. Available online at http://cran.r-project.org/web/packages/SoDA/.

Cottenie, K. 2005. Integrating environmental and spatial processes in ecological community dynamics. Ecol. Lett. **8**(11): 1175–1182. doi:10.1111/j.1461-0248.2005.00820.x. PMID:21352441.

Cottenie, K., Michels, E., Nuytten, N., and De Meester, L. 2003. Zooplankton metacommunity structure: regional versus local processes in highly interconnected ponds. Ecology, **84**(4): 991–1000. doi:10.1890/0012-9658(2003)084[0991:ZMSRVL]2.0.CO;2.

Davidson, T.A., Sayer, C.D., Perrow, M.R., Bramm, M., and Jeppesen, E. 2007. Are the controls of species composition similar for contemporary and sub-fossil cladoceran assemblages? A study of 39 shallow lakes of contrasting trophic status. J. Paleolimnol. **38**(1): 117–134. doi:10.1007/s10933-006-9066-x.

Declerck, S.A.J., Coronel, J.S., Legendre, P., and Brendonck, L. 2010. Scale dependency of processes structuring metacommunities of cladocerans in temporary pools of High-Andes wetlands. Ecography, **34**(2): 296–305. doi:10.1111/j.1600-0587.2010.06462.x.

DeSellas, A.M., Paterson, A.M., Sweetman, J.N., and Smol, J.P. 2008. Cladocera assemblages from the surface sediments of south-central Ontario (Canada) lakes and their relationships to measured environmental variables. Hydrobiologia, **600**(1): 105–119. doi:10.1007/s10750-007-9180-4.

Dodson, S.I., and Frey, D.G. 2001. Cladocera and other Branchiopoda. *In* Ecology and classification of North American freshwater invertebrates. *Edited by* J.H. Thorpe and A.P. Covich. Academic Press, New York. pp. 723–786.

Dray, S., Legendre, P., and Blanchet, G. 2009. packfor: forward selection with permutation. R package version 0.0-7/r58. Available from http://r-forge.r-project.org/projects/sedar/.

Dray, S., Legendre, P., and Peres-Neto, P.R. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol. Model. **196**(3–4): 483–493. doi:10.1016/j.ecolmodel.2006.02.015.

Frey, D.G. 1986. Cladocera analysis. *In* Handbook of palaeoecology and palaeohydrology. *Edited* by B.E. Berglund. Wiley, New York. pp. 667–692.

Frey, D.G. 1982. The reticulated species of *Chydorus* (Cladocera, Chydoridae): two new species with suggestion of convergence. Hydrobiologia, **93**(3): 255–279. doi:10.1007/BF00012335.

Frey, D.G. 1960. The ecological significance of cladoceran remains in lake sediments. Ecology, **41**(4): 684–699. doi:10.2307/1931802.

Garcia, E.A., and Mittelbach, G.G. 2008. Regional coexistence and local dominance in *Chaoborus*: species sorting along a predation gradient. Ecology, **89**(6): 1703–1713. doi:10.1890/07-0737.1. PMID:18589534.

Glew, J. 1988. A portable extruding device for close interval sectioning of unconsolidated core samples. J. Paleolimnol. **1**(3): 235–239. doi:10.1007/BF00177769.

Gray, D.K., and Arnott, S.E. 2011. Does dispersal limitation impact the recovery of zooplankton communities damaged by a regional stressor? Ecol. Appl. **21**(4): 1241–1256. doi:10.1890/10-0364.1.

Gray, D.K., and Arnott, S.E. 2009. Recovery of acid damaged zooplankton communities: measurement, extent, and limiting factors. Environ. Rev. **17**: 81–99. doi:10.1139/A09-006.

Havel, J.E., and Shurin, J.B. 2004. Mechanisms, effects, and scales of dispersal in freshwater zooplankton. Limnol. Oceanogr. **49**(4, part 2): 1229–1238. doi:10.4319/lo.2004.49.4_part_2.1229.

Hessen, D.O., Faafeng, B.A., and Andersen, T. 1995. Competition or niche segregation between *Holopedium* and *Daphnia*: empirical light on abiotic key parameters. Hydrobiologia, **307**(1–3): 253–261. doi:10.1007/BF00032019.

Jeppesen, E., Leavitt, P., De Meester, L., and Jensen, J.P. 2001. Functional ecology and palaeolimnology: using cladoceran remains to reconstruct anthropogenic impact. Trends Ecol. Evol. **16**(4): 191–198. doi:10.1016/S0169-5347(01)02100-0. PMID:11245942.

Keller, W., and Pitblado, J.R. 1984. Crustacean plankton in northeastern Ontario lakes subjected to acidic deposition. Water Air Soil Pollut. **23**(3): 271–291. doi:10.1007/BF00283204.

Keller, W., and Yan, N.D. 1998. Biological recovery from lake acidification: zooplankton communities as a model of patterns and processes. Restor. Ecol. **6**(4): 364–375. doi:10.1046/j.1526-100X.1998.06407.x.

Keller, W., Yan, N.D., Somers, K.M., and Heneberry, J.H. 2002. Crustacean zooplankton communities in lakes recovering from acidification. Can. J. Fish. Aquat. Sci. **59**(4): 726–735. doi:10.1139/f02-042.

Keller, W. 2009. Limnology in northeastern Ontario: from acidification to multiple stressors. Can. J. Fish. Aquat. Sci. **66**(7): 1189–1198. doi:10.1139/F09-080.

Korhola, A., and Rautio, M. 2001. Cladocera and other branchiopod crustaceans. *In* Tracking environmental change using lake sediments. Vol. 4. Zoological indicators. *Edited by* J.P. Smol, H.J.B. Birks, and W.M. Last. Kluwer Academic Publishers, Dordrecht, the Netherlands. pp. 4–41.

Korosi, J.B., Paterson, A.M., DeSellas, A.M., and Smol, J.P. 2008. Linking mean body size of pelagic Cladocera to environmental variables in Precambrian Shield lakes: a paleolimnological approach. J. Limnol. **67**: 22–34.

Kurek, J., Cwynar, L.C., Weeber, R.C., Jeffries, D.S., and Smol, J.P. 2010*a*. Ecological distributions of *Chaoborus* species in small, shallow lakes from the Canadian Boreal Shield ecozone. Hydrobiologia, **652**(1): 207–221. doi:10.1007/s10750-010-0333-5.

Kurek, J., Korosi, J.B., Jeziorski, A., and Smol, J.P. 2010*b*. Establishing reliable minimum count sizes for cladoceran subfossils sampled from lake sediments. J. Paleolimnol. **44**(2): 603–612. doi:10.1007/s10933-010-9440-6.

Leavitt, P.R., Carpenter, S.R., and Kitchell, J.F. 1989. Whole-lake experiments: the annual record of fossil pigments and zooplankton. Limnol. Oceanogr. **34**(4): 700–717. doi:10.4319/lo.1989.34.4.0700.

Legendre, P., and Legendre, L. 1998. Numerical ecology. 2nd English ed. Elsevier Science BV, Amsterdam, the Netherlands.

Legendre, P., and Gallagher, E.D. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia, **129**(2): 271–280. doi:10.1007/s004420100716.

Legendre, P., Borcard, D., and Peres-Neto, P.R. 2005. Analyzing beta diversity: partitioning the spatial variation of community composition data. Ecol. Monogr. **75**(4): 435–450. doi:10.1890/05-0549.

Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M., and Gonzalez, A. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecol. Lett. **7**(7): 601–613. doi:10.1111/j.1461-0248.2004.00608.x.

Louette, G., and De Meester, L. 2005. High dispersal capacity of cladoceran zooplankton in newly founded communities. Ecology, **86**(2): 353–359. doi:10.1890/04-0403.

Minns, C.K., Moore, J.E., Shuter, B.J., and Mandrak, N.E. 2008. A preliminary national analysis of some key characteristics of Canadian lakes. Can. J. Fish. Aquat. Sci. **65**(8): 1763–1778. doi:10.1139/F08-110.

Moore, M.V., Yan, N.D., and Pawson, T. 1994. Omnivory of the larval phantom midge (*Chaoborus* spp.) and its potential significance for freshwater planktonic food webs. Can. J. Zool. **72**(11): 2055–2065. doi:10.1139/z94-275.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., and Wagner, H. 2010. vegan: community ecology package. R package version 1.17-4. Available from http://cran.r-project.org/web/packages/vegan/index.html.

Peres-Neto, P.R., Legendre, P., Dray, S., and Borcard, D. 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology, **87**(10): 2614–2625. doi:10.1890/0012-9658(2006)87[2614:VPOSDM]2.0.CO;2. PMID: 17089669.

Pinel-Alloul, B., Niyonsenga, T., and Legendre, P. 1995. Spatial and environmental components of freshwater zooplankton structure. Ecoscience, **2**: 1–19.

Quinlan, R., and Smol, J.P. 2010. The extant *Chaoborus* assemblage can be assessed using subfossil mandibles. Freshw. Biol. **55**(12): 2458–2467. doi:10.1111/j.1365-2427.2010.02476.x.

R Development Core Team. 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available from http://www.R-project.org.

Riessen, H.P. 1999. *Chaoborus* predation and delayed reproduction in *Daphnia*: a demographic modeling approach. Evol. Ecol. **13**(4): 339–363. doi:10.1023/A:1006715120109.

Rowe, J.S. 1972. Forest regions of Canada. Canadian Forest Service, Ottawa, Ont. Publ. 1300.

Saros, J.E. 2009. Integrating neo- and paleolimnological approaches to refine interpretation of environmental change. J. Paleolimnol. **41**(2): 243–252. doi:10.1007/s10933-008-9237-z.

Schindler, D.W. 2001. The cumulative effects of climate warming and other human stresses on Canadian freshwater in the new millennium. Can. J. Fish. Aquat. Sci. **58**(1): 18–29. doi:10.1139/f00-179.

Shurin, J.B. 2000. Dispersal limitation, invasion resistance, and the structure of pond zooplankton communities. Ecology, **81**(11): 3074–3086. doi:10.1890/0012-9658(2000)081[3074:DLIRAT]2.0.CO;2.

Shurin, J.B., Cottenie, K., and Hillebrand, H. 2009. Spatial autocorrelation and dispersal limitation in freshwater organisms. Oecologia, **159**(1): 151–159. doi:10.1007/s00442-008-1174-z. PMID:18941791.

Smirnov, N.N. 1974. Fauna of the U.S.S.R., Crustacea. Vol. 1: Chydoridae. Israel Program for Scientific Translations, Jerusalem.

Smol, J.P. 2008. Pollution of lakes and rivers: a paleoenvironmental perspective. 2nd ed. Blackwell Publishing, Oxford, UK.

Smol, J.P. 2010. The power of the past: using sediments to track the effects of multiple stressors on lake ecosystems. Freshw. Biol. **55**: 43–59. doi:10.1111/j.1365-2427.2009.02373.x.

Sweetman, J.N., and Smol, J.P. 2006. Reconstructing fish populations using *Chaoborus* (Diptera: Chaoboridae) remains — a review. Quat. Sci. Rev. **25**: 2013–2023. doi:10.1016/j.quascirev.2006.01.007.

Sweetman, J.N., Rühland, K.M., and Smol, J.P. 2010. Environmental and spatial factors influencing the distribution of cladocerans in lakes across the central Canadian Arctic treeline region. J. Limnol. **69**: 1–12.

Szeroczyńska, K., and Sarmaja-Korjonen, K. 2007. Atlas of subfossil Cladocera from central and northern Europe. Friends of the Lower Vistula Society, Świecie.

Telford, R.J., Vandvik, V., and Birks, H.J.B. 2006. Dispersal limitations matter for microbial morphospecies. Science, **312**(5776): 1015. doi:10.1126/science.1125669. PMID:16709777.

Urabe, J., Elser, J.J., Kyle, M., Yoshida, T., Sekino, T., and Kawabata, Z. 2002. Herbivorous animals can mitigate unfavourable ratios of energy and material supplies by enhancing nutrient recycling. Ecol. Lett. **5**(2): 177–185. doi:10.1046/j.1461-0248.2002.00303.x.

Uutala, A.J. 1990. *Chaoborus* (Diptera: Chaoboridae) mandibles — paleolimnological indicators of the historical status of fish populations in acid-sensitive lakes. J. Paleolimnol. **4**(2): 139–152. doi:10.1007/BF00226321.

Valois, A., Keller, W.B., and Ramcharan, C. 2010. Abiotic and biotic processes in lakes recovering from acidification: the relative roles of metal toxicity and fish predation as barriers to zooplankton re-establishment. Freshw. Biol. **55**(12): 2585–2597. doi:10.1111/j.1365-2427.2010.02488.x.

Wagner, H.H., and Fortin, M.-J. 2005. Spatial analysis of landscapes: concepts and statistics. Ecology, **86**(8): 1975–1987. doi:10.1890/04-0914.

Walseng, B., Yan, N.D., and Schartau, A.K. 2003. Littoral microcrustacean (Cladocera and Copepoda) indicators of acidification in Canadian Shield lakes. Ambio, **32**(3): 208–213. PMID: 12839197.

Yan, N.D., Keller, W., Pitblado, J.R., and Mackie, G.L. 1988. *Daphnia–Holopedium* relationships in Canadian Shield lakes ranging in acidity. Verh. Int. Ver. Theor. Angew. Limnol. **23**: 252–257.

Prediction of biomass in Norwegian fish farms

**Anders Løland, Magne Aldrin, Gunnhildur Högnadóttir Steinbakk,
Ragnar Bang Huseby, and Jon Arne Grøttum**

Abstract: We have constructed a statistical model to forecast, with uncertainty, the stock of Norwegian farmed Atlantic salmon (*Salmo salar*). The model provided good predictions of future biomass of Norwegian farmed salmon and can also be used to perform "what-if" analysis exploring the impact of varying scenarios for stocking and slaughtering. The model is based on the number of fish in each mass class (0–1, 1–2, …, 10+ kg) and their average mass. The model, which is related to standard size-structured models, computes the number of fish growing into the next mass class the next month and the number of fish remaining in the same mass class. In addition, the number of new fish stocked, fish lost, slaughtered, and wasted, as well as the sea temperature related to the growth, were modelled. All the model parameters were estimated based on monthly data from 2002 to 2007, and the model was validated statistically. Any animal production involving cycles may benefit from this forecasting tool.

Résumé : Nous avons construit un modèle statistique pour prédire, avec l'incertitude, le stock des saumons atlantiques (*Salmo salar*) norvégiens d'élevage. Le modèle fournit de bonnes prédictions de la biomasse future des saumons norvégiens d'élevage et peut aussi servir à faire une analyse de simulation (« qu'arrive-t-il si? ») qui explore l'impact de divers scénarios d'empoissonnement et de récolte. Le modèle est basé sur le nombre de poissons dans chaque classe de masse (0–1, 1–2, …, 10+ kg) et leur masse moyenne. Le modèle, qui est apparenté aux modèles standard structurés en fonction de la taille, calcule le nombre de poissons qui passent par croissance dans la classe de masse supérieure le mois suivant et le nombre de poissons qui demeurent dans la même classe de masse. De plus, il y a modélisation du nombre de nouveaux poissons empoissonnés, des poissons perdus, tués et gaspillés, de même que de la température de la mer en relation avec la croissance. Tous les paramètres du modèle ont été estimés à partir de données mensuelles de 2002 à 2007 et le modèle a été validé statistiquement. Cet outil de prédiction peut être utile pour toute production animale comportant des cycles.

[Traduit par la Rédaction]

Introduction

Thanks to its extraordinary geographical characteristics, Norway became the first country to actively promote the development of salmon farming. Today, Norwegian interests play an important role in global salmon farming. The ongoing production is performed in sea-based cages. Cage culture production of Atlantic salmon (*Salmo salar*) in the sea has expanded and intensified considerably over the years and in 2009 amounted to 865 000 tonnes. The production is run in about 700 sites along the coast, owned by approximately 160 different commercial companies of different sizes.

As one of the major producers of Atlantic salmon, the Norwegian fish farming industry meets variation in the global market demand for salmon, as well as increased international competition and price volatility. To minimize the fluctuations, it is important for the producers to have knowledge about the expected development of future production and demand. The demand can be influenced by timing or scaling of marketing campaigns. If information about expected production was available, the individual producers could adapt their production plans, for instance by changing the production regime, whereas the fish farming industry on a national level could develop improved production strategies by changing the time for stocking or slaughtering.

The cycle for producing salmon is rather predetermined. After new (young) fish have been stocked, the fish are usually slaughtered after 1½ to 3 years. The production cycle involves systematic fluctuations in biomass and quantity of slaughtered fish. When the new fish have been stocked, the biomass of slaughtered fish is to some degree predictable at a horizon of up to 2 years, since the growth of farmed fish to a large extent is systematic.

The production cyclicity is common among other farmed animals, such as cattle or hog production. This cyclicity and its relationship with the market have been studied for a long time (Russell 1929) and are still debated (Crespi et al. 2010). Recent studies suggest that market timing influences the cattle cycle (Hamilton and Kastens 2000). As Allen (1994, p. 81) points out, "there has been an overemphasis on explanation, and little interest in the predictive power of models" for agricultural production.

Received 14 January 2011. Accepted 9 May 2011. Published at www.nrcresearchpress.com/cjfas on 22 August 2011.
J2011-0050

Paper handled by Associate Editor Terrance J. Quinn II.

A. Løland, M. Aldrin, G.H. Steinbakk, and R.B. Huseby. Norwegian Computing Center, P.O. Box 114 Blindern, NO-0314 Oslo, Norway.
J.A. Grøttum. Norwegian Seafood Federation, P.O. Box 1214 Pirsenteret, NO-7462 Trondheim, Norway.

Corresponding author: A. Løland (e-mail: anders.loland@nr.no).

Can. J. Fish. Aquat. Sci. **68**: 1420–1434 (2011) doi:10.1139/F2011-078 Published by NRC Research Press

The market for farmed salmon is growing, but is less mature than many other agricultural markets and has experienced imperfections because of trade conflicts (Kinnucan and Myrland 2005) and outbreaks of fish diseases. In any case, the increased production of farmed salmon has reduced wild salmon prices (Guillotreau 2004; Asche et al. 2005).

Until now, no reliable tool for forecasting future production on an aggregated level has been available. This is probably due to the lack of coherent data. Our aim is to present a prediction model for regional and national standing biomass. Such a model can also can be used to investigate consequences of changing production strategies.

Since 2002, production data from all Norwegian fish farms have been electronically collected. Monthly data, including the existing stock, stocking of new fish, and slaughtering, are available on a regional level. The data are given as numbers and mean mass of fish in the standing stock within mass classes of 1 kg resolution (0–1, 1–2, …, 9–10, 10+ kg). Accurate age information is lacking.

Since the data are divided into mass classes, it is natural to model them by size-structured models (Hilborn and Mangel 1997; Tuljapurkar and Caswell 1997; Quinn and Deriso 1999). Rizzo and Spagnolo (1996) described optimal management in a single-farm model for European sea bass (*Dicentrarchus labrax*), based on mass classes and biological submodels. Results based on different sea temperatures, harvesting, feeding, and stocking strategies are presented. Gangnery et al. (2001) investigated the production of oysters, where the oysters grow individually between mass classes, and mortality, harvesting, and seeding are included. Halachmi et al. (2005) studied how to optimize the management of a single (or a few) fish farm(s), and Bjørndal (1988) investigated optimal harvesting of farmed fish based on an adapted Beverton–Holt model for a year class of fish. In work related to farmed salmon, Kumbhakar and Tveterås (2003) studied the risk preferences of Norwegian salmon farmers, while Bjørndal (1990) described salmon aquaculture production and harvesting from an economical viewpoint.

Many of these models (Rizzo and Spagnolo 1996; Gangnery et al. 2001) are stochastic on the individual level, but deterministic on the population level. Their parameters are found from the literature, estimated on different data sets, or determined more or less subjectively, as is also the case in Bjørndal (1988). There can be good reasons for this approach. For example, the data can be incomplete, or parameters like the natural mortality can be nonidentifiable. Simulations from the models are presented, but the results are not compared with or validated against the actual data in a thorough analysis. Moreover, the uncertainty in parameters and predictions of the population is more or less ignored.

We propose a dynamic, statistical model that is related to, but not equal to, standard size-structured models. One difference is that the proposed model takes into account also the average mass in each size class in addition to the number of fish. Furthermore, it is constructed such that all unknown parameters can be estimated from one coherent data set, and prediction uncertainty is an inherent part of the model.

The starting point of the model is the abundance and the average mass of the fish in each mass class. Based on this and among others a growth function, depending on mass class, sea temperature, seasonality, and light conditions, the model computes per month how many fish that remain in their mass class and how many that grow into the next mass class the following month. In addition, we modelled the number of new fish stocked, lost (dead and escaped), slaughtered, and wasted (downgraded slaughter fish), learning from the production strategies of earlier years. We demonstrate and justify the predictive ability of the model and describe how "what-if" scenarios can be made.

Materials and methods

Data

Production data from Norwegian fish farms have been collected into the database Havbruksdata since 2002. The data are monthly observations of abundance and standing biomass in each of 11 mass classes: $v = 0$: 0–1 kg, $v = 1$: 1–2 kg, …, $v = 9$: 9–10 kg, $v = 10$: 10+ kg. When both the numbers and biomass are observed, the average mass in each mass class is implicitly given as the biomass divided by the number of fish. In addition, the monthly number of fish stocked (or inserted), lost (died and escaped), slaughtered, and wasted (downgraded slaughter fish), as well as the gutted slaughtered biomass, are known in each mass class. All these data are aggregated into three regions: (*i*) Southern, (*ii*) Mid, and (*iii*) Northern Norway. The data are modelled on a regional basis, but results are aggregated further for Norway as a whole, and all data and results presented here are for Norway unless otherwise stated.

An overview of the various types of data is given (Table 1). For slaughtered fish, we distinguish between the full mass, simply denoted slaughter mass (V_t^S) and gutted mass (V_t^G). The slaughter mass, which is unobserved, is comparable to the mass of the standing stock, whereas the observable gutted mass is lower. The monthly distribution of the data (for Norway as a whole) over a year gives an impression of the seasonal variations (Table 2). Note that the fish are usually stocked around May and some in September and October, while the number of lost fish peaks during the summer. For an overview of the mass class distribution, a summary in absolute numbers per mass class is shown (Table 3). The average number of fish stocked each month is 19.4 million. The corresponding number of fish in mass class 0 is 93.2 million, which is far below 19.4 million times 12 months, since the fish grow into mass class 1 after a few months and some fish die each month (1.7% of the stock in mass class 0 according to the table). Time plots of some of the data described in the Results section complement the picture.

The number of daylight hours D_t in month t partly explains the growth in that month (Boeuf and Le Bail 1999). We used equations from the National Oceanic and Atmospheric Administration, based on work by Meeus (1991), to compute the number of daylight hours for a midpoint in the region for the 15th of each month. For example, in Finnmark in Northern Norway, there are no daylight hours in December and 24 daylight hours (midnight sun) in June.

The growth does also depend on the monthly sea temperature ST_t, averaged over each reporting fish farm in each region, and collected from the database Havbruksdata.

Method overview

The model presented below is first applied to each of the

Table 1. Overview of data and notation.

(*a*) Total numbers and numbers per mass class and month *t*.*

Biomass	No. of fish	Mass per fish	Description
B_t†	N_t†	$V_t^{B\dagger}$	Standing stock
B_t^L	$N_t^{L\dagger}$	V_t^L	Lost
B_t^S	$N_t^{S\dagger}$	V_t^S	Slaughtered
$B_t^{G\dagger}$	$N_t^{S\dagger}$	$V_t^{G\dagger}$	Gutted (slaughtered)
B_t^W	$N_t^{W\dagger}$	V_t^W	Wasted
B_t^R	$N_t^{R\dagger}$	V_t^R	Lost, slaughtered, and wasted
B_t^I	$N_t^{I\dagger}$	V_t^I	Stocked (in mass class 0)
$B_t(v)$†	$N_t(v)$†	$V_t(v)$†	At time *t* in mass class *v*
	$N_t(v, v-1)$	$V_t(v, v-1)$	At time *t* in mass class *v*, who were in mass class *v* – 1 at time *t*

(*b*) Other quantities.

	Description
ST_t†	Sea temperature
D_t†	Number of daylight hours
$f_{t,v}$	Growth function

*$B_t = \sum_{v=0}^{10} B_t(v)$, $N_t = \sum_{v=0}^{10} N_t(v)$, $V_t^B = B_t/N_t$, etc.

†Observable quantities.

Table 2. Distribution of Norwegian farmed salmon data per month (all data shown as percentage of yearly quantities), computed as sample averages over the data period 2002–2007.

Month	N_t	B_t	N_t^L	N_t^S	B_t^G	N_t^I
Jan.	7.7	8.5	6.3	7.3	7.6	0.4
Feb.	7.3	8.1	5.2	6.7	7.0	0.4
Mar.	7.0	7.6	5.2	8.0	8.2	2.0
Apr.	7.3	7.3	6.2	7.5	7.3	12.0
May.	8.7	7.2	9.0	8.2	8.1	29.5
June	9.0	7.2	13.3	8.4	8.2	13.4
July	8.7	7.8	11.2	7.6	7.3	3.7
Aug.	8.6	8.6	10.1	7.9	7.6	4.5
Sept.	8.9	9.2	9.0	8.8	8.6	13.5
Oct.	9.3	9.6	8.5	9.5	9.6	14.3
Nov.	9.1	9.6	8.5	10.2	10.6	5.1
Dec.	8.6	9.2	7.6	10.0	10.0	1.1
Total	100	100	100	100	100	100

Note: N_t, number of fish in the standing stock; B_t, standing biomass; N_t^L and N_t^S, number of fish lost and slaughtered, respectively; B_t^G, gutted slaughtered biomass; N_t^I, number of stocked fish.

three regions separately. Results for Norway as a whole are then computed by aggregating the results over the regions.

The model contains five submodels for monthly values of (*i*) standing stock distributed among mass classes, (*ii*) stocked number of fish, (*iii*) loss, (*iv*) slaughter and waste, and (*v*) sea temperature. The standing stock model is presented in detail below. The other models are outlined in a subsequent section, except for the model for sea temperature, which is described in Appendix A. Details for all the five submodels are provided (Appendices A, C, and D), as well as an overview (Fig. 1).

Generally, the choice of models and the inclusion of linear, nonlinear, and seasonal effects were based on the available literature, analyses of the data, and the resulting model's predictive performance.

Model for standing stock

First, we go through the model for the standing stock. The average mass and the number of fish in each mass class are the key ingredients. For a given growth function, and based on the principle of balance of numbers and biomass, we compute how many fish that remain in their mass class and how many that grow into the next from one month to another. If the average mass of the fish in a mass class is close to the upper boundary of that mass class, many fish will grow into the next mass class. Similar computations are done for the mass in each mass class.

Since individual fish are not observed, we assume that all fish in a mass class *v* have the same growth factor $f_{t,v}$ from time (month) *t* to *t* + 1 and that negative growth is impossible, i.e., $f_{t,v} \geq 0$. The growth is a function of (*i*) mass class, (*ii*) sea temperature, (*iii*) the number of daylight hours, and (*iv*) season. The seasonal component accounts for additional seasonal variations that are not captured by sea temperature and daylight hours. The growth model is then

$$f_{t,v} = 1 + \exp(\eta_{t,v})$$

Table 3. Summary of Norwegian farmed salmon data per mass class (average quantities per month, computed as sample averages over the data period 2002–2007).

Mass class	N_t	B_t	$N_t^L(\%)$	$N_t^S(\%)$	B_t^O	N_t^I
0	93.2	38.2	1.7	0.2	0.1	19.4
1	44.5	64.9	0.8	0.4	0.3	—
2	27.6	68.0	0.5	1.2	0.9	—
3	21.3	74.2	0.6	5.9	5.0	—
4	16.6	74.0	0.7	24.5	19.3	—
5	7.7	41.6	0.6	38.8	16.5	—
6	1.9	12.2	0.5	38.6	4.7	—
7	0.4	2.7	0.6	38.0	1.0	—
8	0.1	1.1	0.9	30.7	0.3	—
9	0.1	0.5	1.3	16.8	0.1	—
10	0.0	0.3	1.7	21.6	0.1	—

Note: Number of fish lost (N_t^L) and slaughtered (N_t^S) is given in percentage of the number of fish in the standing stock (N_t) in each mass class. Biomass (B_t and B_t^O) is given in 1000 tonnes; number of fish (N_t and N_t^I) is given in millions of fish.

Fig. 1. Overview of data and models for salmon abundance and biomass in Norwegian fish farms.

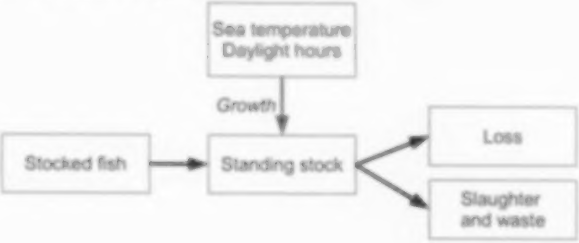
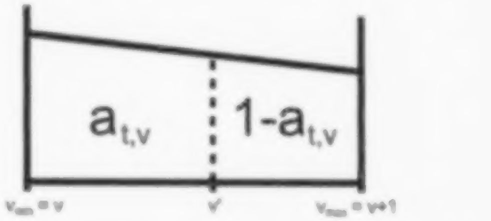


Fig. 2. The distribution of farmed salmon in one mass class. The parameter $a_{t,v}$ is the proportion of fish that remains in the mass class, and $1 - a_{t,v}$ is the proportion of fish that grows into the next mass class.



where the linear predictor $\eta_{t,v}$ is a quadratic function of the sea temperature ST_t and the number of daylight hours D_t. The quadratic terms were included to allow for nonlinear effects, since the salmon does not grow linearly with ST_t and D_t.

The seasonal component is handled by a pair of sine and cosine functions with a period of 1 year. This gives

$$(1)\quad \eta_{t,v} = \beta^{f}_{0,v} + \beta^{f}_{1}\mathrm{ST}_t + \beta^{f}_{2}\mathrm{ST}_t^2 + \beta^{f}_{3}D_t + \beta^{f}_{4}D_t^2 + \beta^{f}_{5}\sin\left(\frac{2\pi t}{12}\right) + \beta^{f}_{6}\cos\left(\frac{2\pi t}{12}\right)$$

Here, the coefficient $\beta^{f}_{0,v}$ is an intercept specific for each mass class v, whereas the other β values are regression coefficients common for all mass classes. To model seasonal variations, we also use the sine and cosine functions throughout the paper.

The regression coefficients are unknown but estimated from historical data by minimizing the prediction errors of monthly numbers and average mass in each mass class (see the next section). They are estimated simultaneously with other unknown parameters introduced later in this section. This is the model for $v \geq 1$. For mass class $v = 0$, the growth is in addition a function of the average mass $V_t(0)$ in mass class $v = 0$ at time t (not shown).

Since all fish in mass class v have the same growth, all fish with a mass above a threshold $v'_{t,v}$ at time t will enter the next mass class (see Fig. 2) at time $t + 1$. The threshold $v'_{t,v}$ is given by $f_{t,v} \cdot v'_{t,v} = v_{\max}$, where $v_{\max} = v + 1$ is the upper boundary of the mass class. This gives

$$v'_{t,v} = \frac{v_{\max}}{f_{t,v}}$$

The number of fish remaining in mass class $N_{t+1}(v, v)$ and the number of fish switching in mass class $N_{t+1}(v + 1, v)$ are given by

$$(2)\quad \begin{aligned} N_{t+1}(v, v) &= a_{t,v} \cdot N_t(v) \\ N_{t+1}(v+1, v) &= (1 - a_{t,v}) \cdot N_t(v) \end{aligned}$$

Here, $a_{t,v}$ is the proportion of fish remaining in the mass class v, and $N_t(v)$ is the number of fish in mass class v in month t.

Assume that the mass of the individual fish in mass class v is distributed according to a probability density $h_{t,v}(w)$ between the boundaries v and $v + 1$. The proportion $a_{t,v}$ is then given by

$$(3)\quad a_{t,v} = \int_{v}^{v'_{t,v}} h_{t,v}(w)\,\mathrm{d}w$$

The true distribution $h_{t,v}(w)$ is unknown, but the average mass $V_t(v)$ of the fish in each mass class v is observed. We assume that the mass distribution $h_{t,v}(w)$ is a beta distribution between v and $v + 1$. The beta distribution has two parameters. We parameterize through the expectation $\mu_{v,t}$ and a dispersal parameter $\gamma_{v,t}$ in the spirit of generalized linear models (McCullagh and Nelder 1989). The parameterization (described in Appendix A) leads to the variance

$$(4) \qquad \mathrm{Var}[h_{v,t}(\cdot)] = \gamma_{v,t} \cdot \mu'_{v,t} \cdot (1 - \mu'_{v,t})$$

where $\mu'_{v,t}$ is between 0 and 1 and given by $\mu_{v,t} = v + \mu'_{v,t}$, i.e., the part of the expectation that exceeds the lower limit v of the mass class. Equation 4 implies that the standard deviation increases with the expected value to the middle of the mass class, where the maximum value is reached. The expectation $\mu_{v,t}$ is given by the mean mass $V_t(v)$, which is observed back in time and can be predicted ahead in time.

For $v \geq 1$, $\gamma_{v,t}$ is a constant β^{γ} independent of time and common for all mass classes. For mass class 0, $\gamma_{v,t}$ varies seasonally according to

$$(5) \qquad \gamma_{0,t} = g\left[\beta_0^{\gamma} + \beta_1^{\gamma}\sin\left(\frac{2\pi t}{12}\right) + \beta_2^{\gamma}\cos\left(\frac{2\pi t}{12}\right)\right]$$

where the β values are unknown regression coefficients. The term $\gamma_{0,t}$ must be between 0 and 1 (see Appendix A), which will be fulfilled by the function

$$(6) \qquad g(x) = \frac{1}{2} + \frac{\arctan(x)}{\pi}$$

The total of four unknown β parameters are estimated from the data by minimizing the prediction errors (see the next section).

We have chosen the beta distribution for $h(\cdot)$ since it is bounded and quite flexible. Note that the proportion $a_{t,v}$ is implicitly a function of both the average mass and the growth (see Fig. 2 for an illustration; more details are found in Appendix A).

The number of fish in mass class v in the next month $t + 1$ is then given by the balance of numbers

$$(7) \qquad N_{t+1}(v) = N_{t+1}(v, v-1) + N_{t+1}(v, v) - N^{\mathrm{R}}_{t+1}(v) + N^{f}_{t+1}(v)$$

which is the sum of the number of fish that switched from mass class $v - 1$ ($N_{t+1}(v, v - 1)$) and those that remained in mass class v ($N_{t+1}(v, v)$), minus the number of fish removed ($N^{\mathrm{R}}_{t+1}(v)$), plus fish stocked ($N^{f}_{t+1}(v)$). The latter is only nonzero for the first mass class $v = 0$, and the number $N^{\mathrm{R}}_{t+1}(v)$ is the sum of fish lost, slaughtered, and wasted.

There is a corresponding, but slightly more complicated, balance of biomass. First, consider those fish that are in mass class v at time t and will remain in the same mass class at time $t + 1$. At time t, the average mass of these fish is given by

$$(8) \qquad V_t^*(v, v) = \frac{\int_{v}^{v'_{t,v}} w \cdot h_{t,v}(w)\,\mathrm{d}w}{\int_{v}^{v'_{t,v}} h_{t,v}(w)\,\mathrm{d}w} = \frac{\int_{v}^{v'_{t,v}} w \cdot h_{t,v}(w)\,\mathrm{d}w}{a_{t,v}}$$

At time $t + 1$, their average mass is increased by the growth factor $f_{t,v}$ to

$$V_{t+1}(v, v) = f_{t,v} \cdot V_t^*(v, v)$$

Correspondingly, consider those fish in mass class v at time t that grow into the next mass class at time $t + 1$. At time t, the average mass of these fish is given by

$$V_t^*(v+1, v) = \frac{\int_{v'_{t,v}}^{v+1} w \cdot h_{t,v}(w)\,\mathrm{d}w}{1 - a_{t,v}}$$

and at time $t + 1$ their average mass has increased to

$$V_{t+1}(v+1, v) = f_{t,v} \cdot V_t^*(v+1, v)$$

To update the average mass, we also need to divide the number of fish removed, $N^{\mathrm{R}}_{t+1}(v)$, into $N^{\mathrm{R}}_{t+1}(v, v)$ and $N^{\mathrm{R}}_{t+1}(v, v-1)$, i.e., into those remaining in the same mass class and those coming from the mass class below at time t. This is done in a similar way as for $N_t(v)$ of the standing stock in eq. 2. Furthermore, we assume that the fish removed and those not removed have the same average mass.

Finally, the average mass of fish in mass class v in the next month $t + 1$ is given by

$$(9) \qquad V_{t+1}(v) = \frac{[N_{t+1}(v, v-1) - N^{\mathrm{R}}_{t+1}(v, v-1)] \cdot V_{t+1}(v, v-1)}{N_{t+1}(v)} + \frac{[N_{t+1}(v, v) - N^{\mathrm{R}}_{t+1}(v, v)] \cdot V_{t+1}(v, v)}{N_{t+1}(v)}$$

Equation 9 above has to be modified for mass class $v = 0$. The quantity $N_{t+1}(v, v-1) - N^{\mathrm{R}}_{t+1}(v, v-1)$ is replaced by the observed number $N_t^{f,obs}$ of stocked fish, and $V_{t+1}(v, v - 1)$ is replaced by the average mass V_t^f of stocked fish, such that

$$V_{t+1}(0) = \frac{N_t^{f,obs} \cdot V_t^f + [N_{t+1}(v, v) - N^{\mathrm{R}}_{t+1}(v, v)] \cdot V_{t+1}(v, v)}{N_{t+1}(v)}$$

The average mass of stocked fish is unknown, but varies over the year. Similar to eq. 5, this average mass is therefore modelled as a seasonal function by

$$(10) \qquad V_t^f = g\left[\beta_0^f + \beta_1^f\sin\left(\frac{2\pi t}{12}\right) + \beta_2^f\cos\left(\frac{2\pi t}{12}\right)\right]$$

where $g(\cdot)$ is given by eq. 6, and the β values are unknown parameters estimated from the data by minimizing the prediction errors (see the next section).

As a summary, the parameters of the model for the standing stock are listed (Table 4).

The proposed model can be compared with the framework of a size-structured or stage-structured model (Quinn and Deriso 1999). In our case the mass classes are the stages, and the model is based on 1-month transitions between the stages. We have imposed the constraint that fish may not grow beyond the next mass class in 1 month. The model for the number of fish described by eqs. 2 and 7 is therefore a special case known as an Usher model (Usher 1996), which (disregarding fish lost, slaughtered, wasted, and stocked) can also be written in matrix form:

$$\mathbf{N}_{t+1} = \mathbf{M}_t\mathbf{N}_t$$

Table 4. Overview of unknown parameters in the model for the standing stock.

Parameter	Parameter group	Eq.	Description
$\beta^{a}_{0,v}$	Growth	1	Intercept growth, mass class v
$\beta^{a}_{1}, \beta^{a}_{2}$	Growth	1	Sea temperature driven growth
$\beta^{a}_{3}, \beta^{a}_{4}$	Growth	1	Daylight hours driven growth
$\beta^{a}_{5}, \beta^{a}_{6}$	Growth	1	Seasonal growth
β^{σ}	Mass distribution within a mass class		Intercept dispersal, mass class $v \geq 1$
β^{σ}_{0}	Mass distribution within mass class 0	5	Intercept dispersal, $v = 0$
$\beta^{\sigma}_{1}, \beta^{\sigma}_{2}$	Distribution within mass class 0	5	Seasonal dispersal, $v = 0$
β^{d}_{0}	Mass of stocked fish	10	Intercept mass
$\beta^{d}_{1}, \beta^{d}_{2}$	Mass of stocked fish	10	Seasonal mass

where N_t is a vector of the number of fish in each mass class. The growth transition matrix $\mathbf{M}_t$ is given as $\mathbf{M}_t(v, v) = a_{t,v}$, $\mathbf{M}_t(v + 1, v) = 1 - a_{t,v}$, where $a_{t,v}$ is given by eq. 3 for mass class v, and all other $\mathbf{M}_t(i, j) = 0$. Note that $\mathbf{M}_t$ is time-varying. Contrary to many of the stage-structured models, we need to model both the mass and the number of fish in each mass class, with a nonlinear connection between the two and based on the unknown mass distribution of fish within each mass class. Our mass model (eq. 9) cannot easily be written in matrix form.

Estimation of model for the standing stock

The unknown parameters from the model for the standing stock are estimated from the historical data by minimizing the prediction errors of monthly numbers and average mass in each mass class. It is essential that the estimated model gives sensible predictions for both abundance and mass of fish, for different mass classes as well as on the short and long term. How these various goals should be balanced is, however, not obvious. Furthermore, it is not obvious which (joint) statistical distribution the numbers and mass of fish follow. We have therefore chosen not to use maximum likelihood or any model-based fitting criterion, but instead to minimize a least-squares-type criterion, which turns out to be relatively robust against modelling error and numerical optimization problems.

This has the unfortunate consequence that measures of precision for the parameters in Table 4 are not readily available. (The submodel parameters are accompanied by precision estimates.) However, our primary concern has been to build a proper forecasting model for noisy data, and the precision parameter of the in-sample fit is not crucial in pursuing the best forecasting model.

First, let $N^{\text{obs}}_{t+k}(v)$ and $V^{\text{obs}}_{t+k}(v)$ denote the observed number and average mass of fish, respectively, in mass class v at time $t + k$. Furthermore, let $\widehat{N}_{t+k|t}(v)$ and $\widehat{V}_{t+k|t}(v)$ denote the corresponding conditional k-step-ahead predictions, conditioned on all observed quantities at time t and on the observed number of removed (lost, slaughtered, wasted) and stocked fish, as well as on the sea temperature and the number of daylight hours at times between $t + 1$ and $t + k$. The following fitting criterion takes into account prediction errors for numbers and average masses for the first seven mass classes and for prediction horizons from 1 month to 1 year:

$$\sum_{k=1}^{12}\left(\sqrt{\sum_{t=1}^{T}\sum_{v=0}^{6}\{w_N[\widehat{N}_{t+k|t}(v) - N^{\text{obs}}_{t+k}(v)]\}^2} + \sqrt{\sum_{t=1}^{T}\sum_{v=0}^{6}w_V(v)[\widehat{V}_{t+k|t}(v) - V^{\text{obs}}_{t+k}(v)]^2}\right) \qquad (11)$$

where $t = 1, 2, \ldots, T$ are months over the data period 2002–2007, and w_N and $w_V(v)$ are weights chosen to give a reasonable balance between mass classes and between numbers and average masses of fish. The weights for numbers are given by $w_N = 1/\overline{N}^{\text{obs}}$, where $\overline{N}^{\text{obs}}$ is the sample mean of the observed number of fish over the entire data period. The first term of the criterion in eq. 11 then consists of a sum of relative prediction errors for the number of fish. The weights for average masses put more emphasis on the dominating mass classes, defined as $w_V(v) = N_t(v)^{\text{obs}}/N_t^{\text{obs}}$. Since data in the upper mass classes ($v = 7 - 10$) are scarce and very variable, these mass classes are ignored in the estimation.

Model for number of fish stocked and other submodels

Typically, a lot of fish are stocked in some months (late spring and early autumn) and very few are stocked in other months. There is, in principle, no upper limit on the number of stocked fish, but a lower limit at 0. The number of fish stocked at month t is modelled as a gamma distribution:

$$N_t^s \sim \text{gamma}(\lambda_t, \sigma_t) \qquad (12)$$

Here, λ_t is the expected value at month t given by

$$\lambda_t = s(t) + \beta_1^{\lambda}\sin\left(\frac{2\pi t}{12}\right) + \beta_2^{\lambda}\cos\left(\frac{2\pi t}{12}\right)$$

where $s(t)$ is a smooth trend over the data period with four unknown parameters (see Appendix F for details), and the β values are unknown regression coefficients expressing seasonal variation. The corresponding standard deviation σ_t is a function of the expected value, given by $\sigma_t = \sigma_0\,(\lambda_t)^{\delta}$, where δ and σ_0 are unknown parameters. In terms of a standard gamma distribution parameterization, $\text{shape}_t = \lambda_t^{2(1-\delta)}/\sigma_0^2$ and $\text{scale}_t = \lambda_t/\text{shape}_t$. This distribution allows for a seasonally varying relative variance (or coefficient of variation).

The models for number of fish lost, slaughtered, and wasted (Appendix C) are more or less similar. Furthermore, there is a model for the gutted mass of slaughtered fish (Appendix D) and a model for the sea temperature (Appendix E).

All the submodels, except for the model for the standing stock, are estimated separately by maximum likelihood (Pawitan 2001) by numerically maximizing the log likelihood or by using standard software for generalized linear models. For each of the submodels (and for each mass class), the parameters are estimated conditional on all other data. We assume, for example, that the number of fish $N_{t-1}(v)$ is known when estimating the loss model (eq. C.1) for $N_t^L(v)$.

Our submodels are richer than what is common in the literature (Rizzo and Spagnolo 1996; Ferreira et al. 1998; Gangnery et al. 2001). The submodels are not deterministic to better represent the actual possible variation between months and years in stocking, loss, slaughtering, and waste. Assuming deterministic submodels, for example, stocking would underestimate the uncertainty in future loss and standing biomass because of uncertain and time-varying loss and hence produce worse predictions.

Predictions with uncertainty

The fitting criterion (eq. 11) we used to estimate the standing stock model involved the conditional k-step-ahead predictions $\widehat{N}_{t+k|t}(v)$ of numbers and $\widehat{V}_{t+k|t}(v)$ of average masses, where we conditioned not only on data known at time t, but also on observed values of stocked and removed fish and sea temperature between time $t + 1$ and $t + k$. However, in an ordinary prediction situation, these additional variables are unknown and must be predicted as well by their respective submodels. To take into account their prediction uncertainties, Monte Carlo simulated values from the submodels are used.

Even if the number of stocked and removed fish and the sea temperatures between time $t + 1$ and $t + k$ were known, there would be a discrepancy between predicted and observed values, because of model and observational error. To take this uncertainty into account, we compute empirical prediction errors from the historical data and add samples from these to the predicted values to get a predictive distribution (Appendix B).

Our procedure may be summarized as follows. To predict future states, we (*i*) condition on the present data known at time t, (*ii*) sample future random numbers between time t + 1 and $t + k$ for every term in the model that follows a distribution (e.g., the gamma distribution for the number of fish stocked), (*iii*) compute the number and mass of fish for the next k months, and (*iv*) add a sample of empirical prediction errors for numbers and average mass. This procedure is repeated B times. The prediction is equal to the sample mean of the B simulations for each future point in time. To distinguish these unconditional predictions from the conditional ones, we use the notation $\widetilde{N}_{t+k|t}(v)$. A 90% prediction interval is correspondingly found from the 5% and 95% quantiles of the simulations.

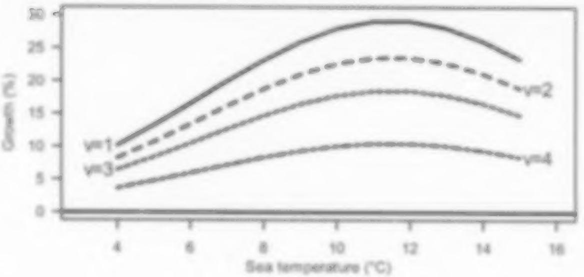
Validation

To demonstrate that the model provides sensible and robust predictions, we validate the predictions by out-of-sample validation. Starting with two years of historical data (2002 and 2003), we estimated parameters based on these data and predicted the next year. For each prediction month, we compared the prediction with the actual data and computed the prediction error. Next, we used 2 years plus 1 month of historical data, 2 years plus 2 months of historical data, and so on. Based on this, we computed the mean relative prediction error k months ahead, given the observation Y_{t+k} of one of the quantities of interest (for instance N_{t+k}), and the corresponding prediction $\widetilde{Y}_{t+k|t}$ at time t:

$$(13)\qquad \frac{1}{T-k-t_1+1}\sum_{t=t_1}^{T-k}\frac{|\widetilde{Y}_{t+k|t}-Y_{t+k}|}{Y_{t+k}}$$

for k = 1–12 months, where t_1 is December 2003.

Fig. 3. Estimated monthly growth for farmed salmon as a function of sea temperature (for daylight hour 15 in April) for four mass classes for Mid Norway.



For comparison, the same criterion (eq. 13) was computed for a naive predictor, which was chosen to be the last observed value. For 1 month ahead prediction, this is the value of the current month.

The naive predictor is expected to perform worse if the seasonality is strong, and therefore the comparison may be unfair, yet it is simple and easy to understand. Since the data are quite stationary, a simple regression model with a linear time trend and seasonal variation could be an alternate and improved naive predictor. However, even if such an empirical predictor can give reasonably good predictions within the present historical data, it will neither be useful in the future if important aspects, such as the stocking strategy, changed, nor in different "what-if" analysis. Therefore, an improved naive predictor is not a realistic alternative to our more causal model, which satisfies the balance of numbers and mass.

Scenarios for stocked fish or slaughtering strategy

The model can also be used for investigating "what-if" scenarios. This means that we replace simulations from one or more of the submodels with certain scenarios for those quantities. For instance, one may be interested in developing a production strategy that gives less seasonal variations than today. This can be investigated by changing stocking or slaughtering strategies according to given scenarios and study the corresponding changes in seasonal patterns for standing stock or gutted slaughtered biomass.

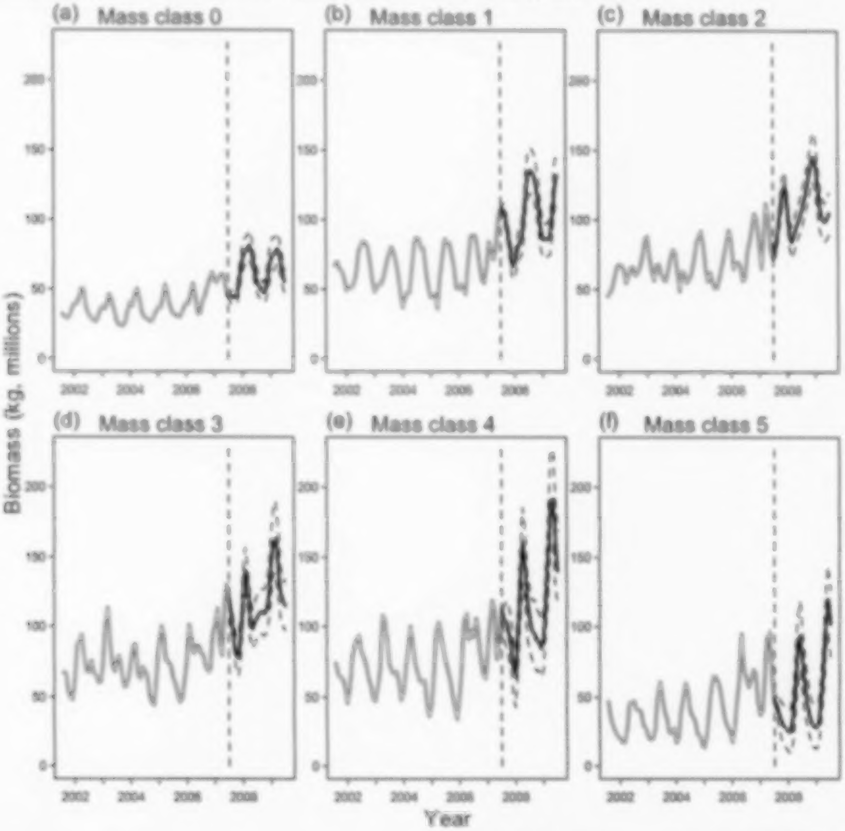
Scenarios can also be useful if the approximate number of smolt to be set out is known some months in advance. Then, using the known number of future stocked fish instead of the predicted numbers from our stocking model may improve the precision of the predictions of the standing stock considerably.

Table 5. Values for of the estimated parameters in the model for the standing stock for each of the three regions (confer with Table 4 for a description of the parameters).

Parameter	Parameter group	Region of Norway		
		Southern	Mid	Northern
$\beta^g_{0,0}, \beta^g_{0,1}$	Growth	–3.9, –3.8	–3.9, –4.1	–4.6, –5.2
$\beta^g_{0,2}, \beta^g_{0,3}$	Growth	–4.2, –4.5	–4.4, –4.6	–5.3, –5.6
$\beta^g_{0,4}, \beta^g_{0,5}$	Growth	–5.3, –4.8	–5.2, –5.6	–6.0, –6.1
β^g_1, β^g_2	Growth	0.22, –0.0077	0.43, –0.019	0.51, –0.021
β^g_3, β^g_4	Growth	0.16, –0.0062	0.040, –0.00092	0.15, –0.0049
β^g_5, β^g_6	Growth	–0.034, –0.19	0.024, –0.060	0.32, –0.026
β^v	Mass distribution	–0.70	–0.61	–0.61
β^v_0	Mass distribution	–0.79	–0.65	–0.81
β^v_1, β^v_2	Mass distribution	–0.11, 0.16	–0.10, 0.079	0.0019, 0.22
β^s_0	Mass of stocked fish	–2.0	–1.7	–2.2
β^s_1, β^s_2	Mass of stocked fish	–0.10, –0.66	–1.3, –0.55	–2.4, 0.44

Note: Some parameters are listed pairwise. For example, for Mid Norway, $\beta^g_{0,0} = -3.9$ and $\beta^g_{0,1} = -4.1$.

Fig. 4. Prediction of standing biomass of farmed salmon for Norway per mass class $B_t(v)$. Historical data (from January 2002): grey lines; predictions (from January 2008): black lines, with 90% prediction intervals shown by dotted lines.



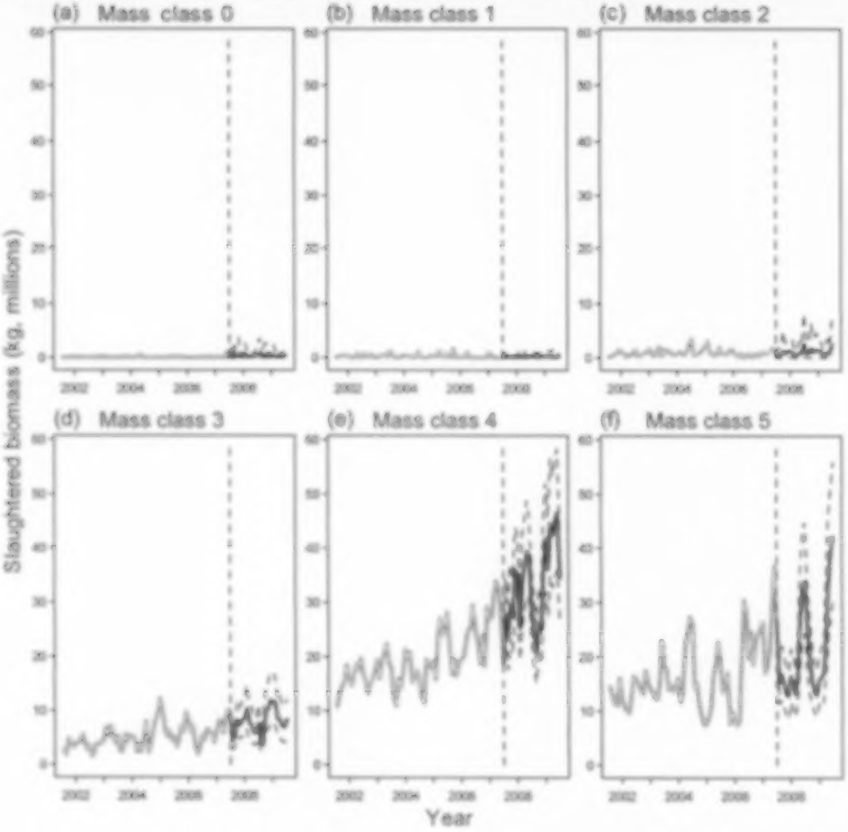
Results

Parameter estimation

The various submodels were estimated for each of the three regions (South, Mid, Northern Norway) separately on the data from 2002 to 2007. The estimated growth as a function of the sea temperature for four mass classes in Mid Norway is displayed (Fig. 3). The relative growth decreases along with increasing mass and has a maximum when the sea temperature is around 11–12 °C.

There are plenty of parameters in the model (but not more than what is justifiable from the amount of data). Many of

Fig. 5. Prediction of slaughtered (gutted) biomass of farmed salmon for Norway per mass class $B^S_l(v)$. Historical data (from January 2002): grey lines; predictions (from January 2008): black lines, with 90% prediction intervals shown by dotted lines.



the parameters are not directly interpretable. Estimates for the most important parameters (the parameters in the model for the standing stock) are supplied for the three regions (Table 5). The intercepts ($\beta^g_{0,0}$, $\beta^g_{0,1}$, …) of the growth model (eq. 1) generally decrease with increasing mass class (in agreement with Fig. 3). For equal sea temperature and number of daylight hours, the growth is generally lower in the north. Both the sea temperature and the number of daylight hours growth parameters imply a concave growth function (β^g_1 and β^g_3 are positive, β^g_2 and β^g_4 are negative) for all three regions. The variance intercept terms (β^V and β^S_0) and the mass of stocked fish intercept term (β^s_0) are all quite similar across the regions (after the transformation; eq. 6). Of the corresponding seasonal fluctuations, the absolute values of the parameters for the seasonally varying mass of stocked fish (β^s_1 and β^s_2) for the Northern region stand out as high.

Predictions with uncertainty

We display two examples of predictions with uncertainty, based on aggregation of regional results, to make predictions for Norway (the predictions in Figs. 4 and 5 are based on B = 1000 simulations). The results are shown for mass classes 0–5, since the upper ones are of marginal interest.

The predicted biomass in each mass class (Fig. 4) is increasing, in line with the historical trend, and approximately proportional to the predicted number of fish (not shown), because the biomass is the product of the number of fish and their average mass. The latter is quite stable over time and is predicted with rather high precision (results not shown).

The predicted slaughtered biomass of gutted fish (Fig. 5) displays a more diverse picture, also because the slaughtered biomass historically has been more variable than the current stock quantities. The slaughtered biomass is quite uncertain because of uncertainty in both numbers and mass, as well as historically changing slaughtering strategies.

Generally, the relative uncertainty is increasing with prediction horizon and mass class, as expected.

Model validation

To investigate the goodness of the model fit, we performed out-of-sample validation of the predictions on the data for the period 2004–2007 (at least 2 years are used for estimating the model). The mean relative prediction error (eq. 13) for the biomass is shown (Fig. 6). As expected, the prediction error generally increases with the prediction horizon and the mass class. The model-based predictions are better than the naive predictor in the short run and approximately equal to the naive predictor 12 months ahead.

The results for the numbers of fish (not shown) were very

Fig. 6. Mean relative prediction error resulting from out-of-sample validation of predictions 1–12 months ahead of the standing biomass B_t of farmed salmon for Norway for mass classes 0–5 (model: black line; naive predictor (last observed value): dotted line).

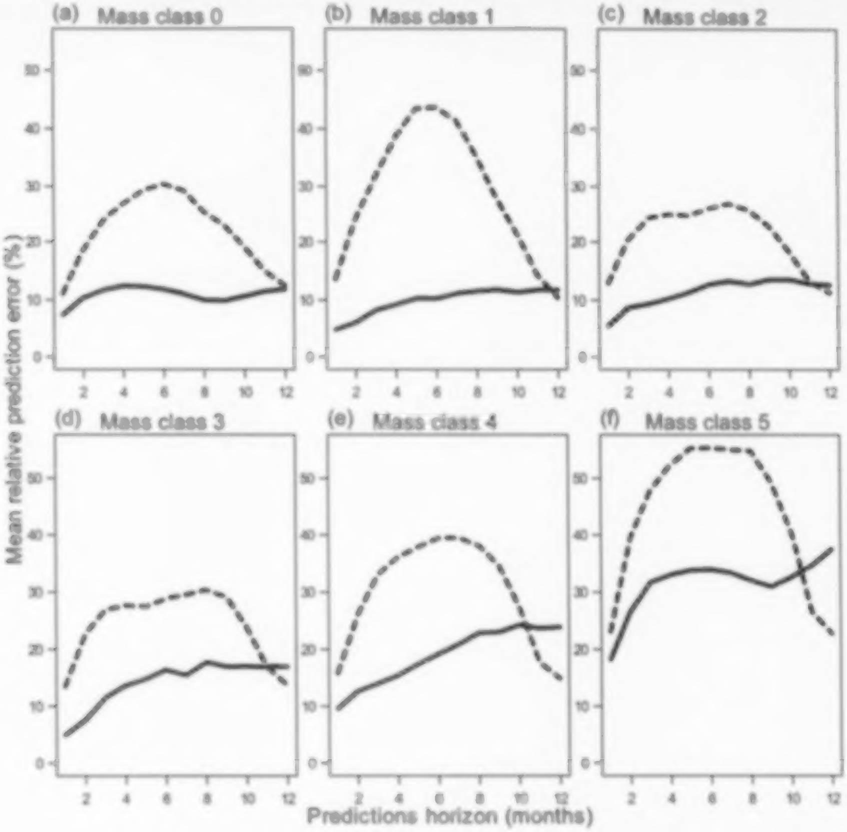
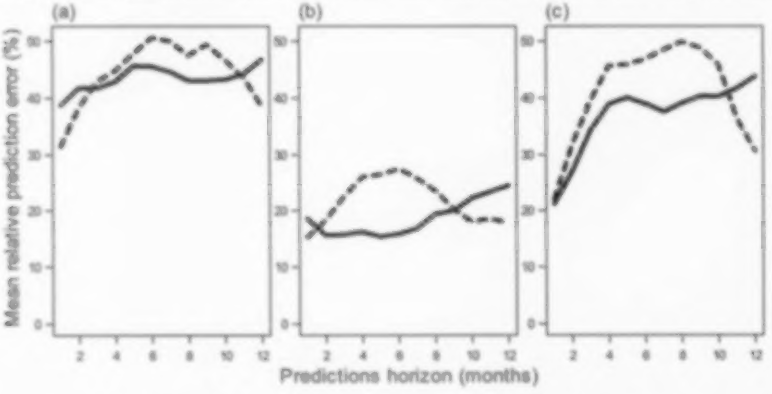


Fig. 7. Mean relative prediction error resulting from out-of-sample validation of predictions 1–12 months ahead of the slaughtered biomass B_t of farmed salmon for Norway for mass classes (from left) 3–5 (model: black line; naive predictor (last observed value): dotted line).

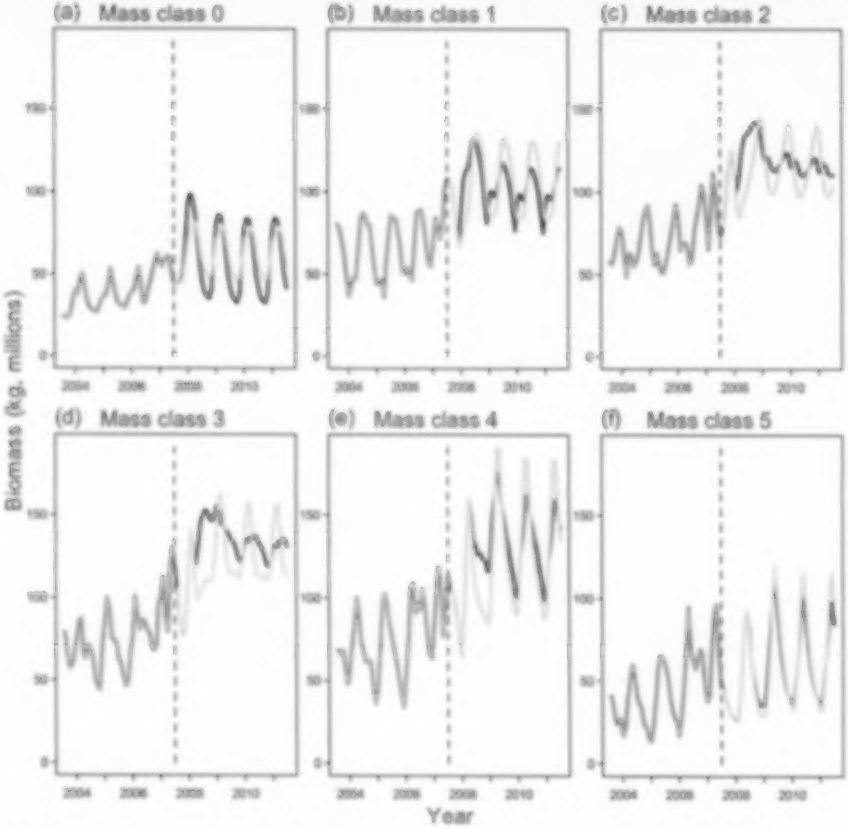


similar. As explained above, this is because the average mass per fish per mass class is both stable over time and predicted rather precisely.

The mean relative prediction error for the slaughtered biomass is presented (Fig. 7). The results for mass classes 0–2 are of marginal interest and therefore not shown here, since most of the slaughtering takes place in mass classes 3–5. Here, the results based on the model are slightly better than the naive predictor, but the prediction error is large (20%–40%) for both predictors and at its lowest (20%) in mass class 4, where most of the biomass is being slaughtered.

We also estimated how often the prediction intervals were violated. The results (not shown here) indicate that the uncertainty is somewhat underestimated.

Fig. 8. Prediction of standing biomass for Norway per mass class $B_t(v)$ for a stocking scenario, where all the farmed salmon were stocked during the spring. Historical data (from January 2004 to January 2008) is shown by grey lines. Predicted biomass after 2008 from Fig. 4 is shown by grey lines. Predicted biomass for the stocking scenario is shown by black lines. Vertical dotted lines indicate January 2008.



"What-if" scenarios for stocking strategy and slaughtering strategy

We performed a scenario analysis on stocking strategy to demonstrate how the model can be used for "what-if" analysis to investigate consequences of potential changes in production strategy.

One problem in the production has been the strong seasonality, which to some extent forces the amount of slaughtered fish to be driven by the supply and not by the demand of fish. Therefore, it is of interest to investigate if other production strategies can give a more stable production over time.

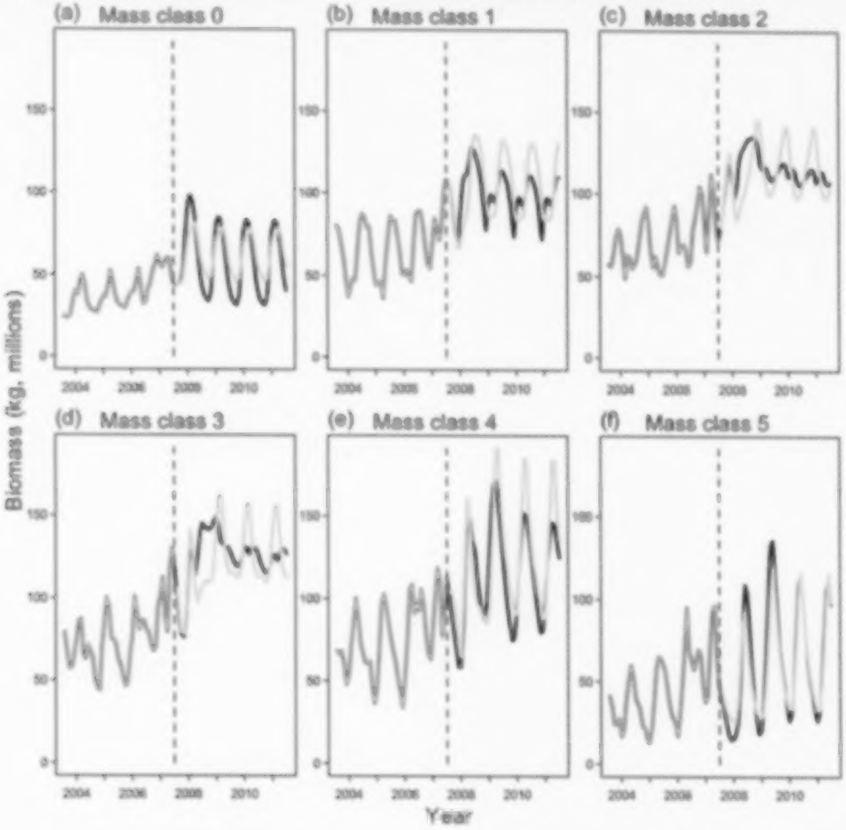
Traditionally, most fish have been stocked during spring and autumn (see Table 2). As a hypothetical alternative to the current practice, we assumed a scenario where all the fish were stocked during the spring. This is an extreme stocking strategy, but provides a good illustration of the model. We compared the results with a scenario where the current stocking strategy was followed as predicted by our stocking submodel (eq. 12). The slaughtering strategy was the same in the two scenarios, given by our slaughter model to represent the current strategy. Predictions for the two scenarios are displayed (Fig. 8), conditioned on the situation in December 2007 (the prediction intervals depicted in Fig. 4 are not shown here). Since the predictions for the first months depend highly on the initial conditions, we produced forecasts up to 4 years ahead to see the long-term effect of the stocking strategy. For mass class 0, the stocking strategy resulted in a more pronounced seasonality. For mass classes 2 and 3, the seasonal variation was reduced a great deal. For mass classes 4 and 5, however, the reduction is less, because of the high number of fish being slaughtered during the autumn.

Slaughtering is not only governed by changing prices. Sometimes there is a need to slaughter to avoid too many fish in the upper mass classes. Our second scenario therefore combines the stocking scenario (Fig. 8) with a slaughtering strategy where (as far as possible) an equal amount of fish is slaughtered each month in mass classes 3–5 (Fig. 9). There is little slaughtering in mass classes 0–2 (Table 3), and we use the submodels for slaughtering from these mass classes. The result (compared with Fig. 8) is quite few changes in mass classes 4 and 5. This demonstrates that when allowing for a stocking strategy, a flat slaughtering strategy is possible with quite few consequences for the standing stock.

Discussion

We have described a statistical model to predict the stock of Norwegian farmed salmon. The model was designed for our unique data set, where production data from all Norwegian fish farms have been electronically collected into the da-

Fig. 9. Prediction of standing biomass for Norway per mass class $B_i(v)$ for a combined stocking and slaughtering scenario, where all the farmed salmon were stocked during the spring, and we slaughter the same amount in mass classes 3–5 each month of the year. Historical data (from January 2004 to January 2008) is shown by grey lines. Predicted biomass after 2008 from Fig. 4 is shown by grey lines. Predicted biomass for the stocking scenario is shown by black lines. Vertical dotted lines indicate January 2008.



tabase Havbruksdata since 2002. The model starts with the number and the mass of fish in a mass class. We computed how many fish that remain in a mass class and how many that grow into the next mass class. In addition, we modelled the number of new fish stocked, lost, slaughtered, and wasted. Based on the statistical validation, we concluded that the model provides sensible predictions with uncertainty of future production of Norwegian farmed salmon. In addition to prediction, the model can be used for "what-if" scenarios, particularly for testing the effect of changed stocking and slaughtering strategies.

The growth rate of individual farmed salmon (from the same age class) depends on their mass, temperature in the sea, light conditions given by geographical location, and amount of feed (Gardeur et al. 2001; Mørkøre and Rørvik 2001). We have chosen to disregard feed consumption to explain growth, since it is difficult to separate the effect of feed consumption from the effects of sea temperature and seasonality. Other factors may impact production, such as a suboptimal environment, reduced fish health, and technological challenges. However, the influence of these factors are not systematic and therefore almost impossible to model and predict. Usually these factors will influence on a smaller part of the production and will therefore not have a major influence on the estimated values.

Our model can be compared with the framework of a size-structured or stage-structured model (Quinn and Deriso 1999). However, we go beyond the standard stage-structured model, since both the number of fish and the average mass of fish in each mass class (stage) are modelled, and we estimated the unknown mass distribution of fish in each mass class to accomplish this.

The parameters of our model were estimated from and validated on one coherent data set. In contrast with similar models in the literature, which usually are deterministic, our submodels are stochastic to better represent the actual variation in stocking, loss, and slaughtering.

One may wonder why the regional approach, where data are aggregated over multiple farms, was used instead of a set of single-farm models. This was not a feasible option for two reasons. (*i*) The data are far from error free. Even though all farmers are obliged to report each month, data from a few farms were missing each month. The proportion of missing farms was known, and the data were scaled up to avoid bias. However, even with all farms reporting their numbers, the numbers themselves are not error free. The impact of these errors was reduced when aggregating over multiple farms. (*ii*) Individual farm data are protected and cannot be used without a written consent.

Forecasting production fluctuations may be an important tool for optimizing the production planning for Norwegian fish farms, especially since large companies represent the majority of the Norwegian production. There is a range of interesting "what-if" scenarios to investigate. We have already studied a stocking and a slaughtering strategy. One could also study the effect of changing framework conditions, for example: (*i*) High prices lead to excess slaughtering in mass class 3, resulting in fewer fish in mass classes 4 and 5 later on. (*ii*) Fish diseases lead to obligatory slaughtering of half the fish in mass class 2 in a particular month. (*iii*) The number of stocked fish is halved one year because of smolt production problems. (*iv*) Increased spring slaughtering is needed to cope with increased demand during the spring.

Any animal production involving cycles may benefit from this tool, since it takes quite some time between the time a producer decides to expand the production and the time those animals have reached their slaughter mass. Both for livestock and farmed fish, the long- and short-term impact of market prices can be built into the model.

The model is, however, a first attempt at predicting the production and can be improved in many ways. Since the balances of number and mass are not always correct, there are inconsistencies in the data. An improved model, where inconsistent data were weighted down, could provide even better predictions.

To achieve that goal and avoid our somewhat ad hoc approach, we advocate the use of Bayesian hierarchical models (Gelman et al. 2004) within a state–space framework (Durbin and Koopman 2001). This allows for seperate modelling of the system process and the data process. The system process is essentially the theoretical model for the standing stock and the submodels. The data process describes the observable quantities, measurement errors, and how the data are connected to the system process. Still, how to properly balance the numbers and mass of fish, as well as the short- and long-term performance of the model, continues to be a challenge.

Acknowledgements

We thank Peder A. Jansen for useful suggestions on drafts of this paper. This research has been funded by The Fishery and Aquaculture Industry Research Fund (FHF), project number 263044, and the Research Council of Norway project "Modern Application driven Statistical Challenges (186951/I30)".

References

Allen, P.G. 1994. Economic forecasting in agriculture. Int. J. Forecast. **10**(1): 81–135. doi:10.1016/0169-2070(94)90052-3.

Asche, F., Guttormsen, A.G., Sebulonsen, T., and Sissener, E.H. 2005. Competition between farmed and wild salmon: the Japanese salmon market. Agric. Econ. **33**(3): 333–340. doi:10.1111/j.1574-0864.2005.00072.x.

Bjørndal, T. 1988. Optimal harvesting of farmed fish. Mar. Resour. Econ. **5**: 139–159.

Bjørndal, T. 1990. The Economics of Salmon Aquaculture. Blackwell Scientific Publications, Oxford, UK.

Boeuf, G., and Le Bail, P.Y. 1999. Does light have an influence on fish growth? Aquaculture, **177**(1–4): 129–152, 3rd International Symposium on Research for Aquaculture — Fundamental and Applied Aspects, Barcelona, Spain, 24–27 August 1997.

Crespi, J.M., Xia, T., and Jones, R. 2010. Market power and the cattle cycle. Am. J. Agric. Econ. **92**(3): 685–697. doi:10.1093/ajae/aap034.

Durbin, J., and Koopman, S.J. 2001. Time series analysis by state space methods. Oxford University Press, Oxford, UK.

Ferreira, J.G., Duarte, P., and Ball, B. 1998. Trophic capacity of Carlingford Lough for oyster culture — analysis by ecological modelling. Aquat. Ecol. **31**(4): 361–378. doi:10.1023/A:1009952729216.

Gangnery, A., Bacher, C., and Buestel, D. 2001. Assessing the production and the impact of cultivated oysters in the Thau lagoon (Mediterrance, France) with a population dynamics model. Can. J. Fish. Aquat. Sci. **58**(5): 1012–1020. doi:10.1139/f01-028.

Gardeur, J.N., Paspatis, M., Gelineau, A., and Boujard, T. 2001. Biostatistical implications of individual variability in growth in rainbow trout and Atlantic salmon. Aquaculture, **195**(1–2): 51–59. doi:10.1016/S0044-8486(00)00542-1.

Gelman, A., Carlin, J.B., Stern, H.S., and Rubin, D.B. 2004. Bayesian data analysis. 2nd edition. Chapman and Hall/CRC, Boca Raton, Fla.

Guillotreau, P. 2004. How does the European seafood industry stand after the revolution of salmon farming: an economic analysis of fish prices. Mar. Policy, **28**(3): 227–233. doi:10.1016/j.marpol.2003.07.001.

Halachmi, I., Simon, Y., Guetta, R., and Hallerman, E.M. 2005. A novel computer simulation model for design and management of re-circulating aquaculture systems. Aquacult. Eng. **32**(3–4): 443–464. doi:10.1016/j.aquaeng.2004.09.010.

Hamilton, S.F., and Kastens, T.L. 2000. Does market timing contribute to the cattle cycle? Am. J. Agric. Econ. **82**(1): 82–96. doi:10.1111/0002-9092.00008.

Hilborn, R., and Mangel, M. 1997. The ecological detective: confronting models with data. Monographs in population biology. Princeton, N.J.

Kinnucan, H.W., and Myrland, O. 2005. Effects of income growth and tariffs on the world salmon market. Appl. Econ. **37**(17): 1967–1978. doi:10.1080/00036840500217523.

Kumbhakar, S.C., and Tveterås, R. 2003. Risk preferences, production risk and firm heterogeneity. Scand. J. Econ. **105**(2): 275–293. doi:10.1111/1467-9442.t01-1-00009.

McCullagh, P., and Nelder, J.A. 1989. Generalized linear models. 2nd edition. Chapman and Hall, London, UK.

Meeus, J. 1991. Astronomical algorithms. Willmann-Bell, Richmond, Va.

Mørkøre, T., and Rørvik, K.A. 2001. Seasonal variations in growth, feed utilisation and product quality of farmed Atlantic salmon (*Salmo salar*) transferred to seawater as 0+smolts or 1+smolts. Aquaculture, **199**(1–2): 145–157. doi:10.1016/S0044-8486(01)00524-5.

Pawitan, Y. 2001. In all likelihood: statistical modelling and inference using likelihood. Oxford University Press, Oxford, UK.

Quinn, T.J., and Deriso, R.B. 1999. Quantitative fish dynamics. Oxford University Press, New York.

Rizzo, G., and Spagnolo, M. 1996. A model for the optimal management of sea bass *Dicentrarchus labrax* aquaculture. Mar. Resour. Econ. **11**: 267–286.

Russell, S.W. 1929. Forecasting hog production and marketing. J. Am. Stat. Assoc. **24**(165): 225–233. doi:10.2307/2277045.

Tuljapurkar, S., and Caswell, M. 1997. Structured-population models in marine, terrestrial, and freshwater systems. Volume 18 of Population and Community Biology Series. Springer, New York.

Usher, M.B. 1996. A matrix approach to the management of renewable resources, with special reference to selection forests. J. Appl. Ecol. 3: 355–367.

Appendix A. The mass distribution in one mass class

We have assumed that the mass w of fish in mass class v is beta distributed between v and $v + 1$. If we write $2 = v + x$, where x is the mass that exceeds the lower limit in the mass class, x is beta distributed between 0 and 1 with density

$$\text{(A.1)}\qquad \begin{aligned} h'(x;p,q) &= \frac{\Gamma(p+q)}{\Gamma(p)\Gamma(q)}x^{p-1}(1-x)^{q-1} \\ &= x^{p-1}(1-x)^{q-1}/B(p,q) \end{aligned}$$

where we use $h'(\cdot)$ to distinguish it from the shifted distribution $h(\cdot)$ between v and $v + 1$. Here, $p > 0$ and $q > 0$ are parameters, $\Gamma(\cdot)$ is the gamma function, and $B(p, q) = \Gamma(p)\Gamma(q)/\Gamma(p + q)$. The relation between p and q and the parameters μ and γ we used in the model for the standing stock are found from

$$\text{Expected value} = \mu = (V_t - v) = \frac{p}{p+q}$$

$$\text{(A.2)}\qquad \text{Variance} = \frac{pq}{(p+q)^2(p+q+1)} = \gamma[\mu(1-\mu)]$$

$$\Downarrow$$

$$\gamma = \frac{1}{p+q+1}$$

The parameter γ is between 0 and 1, since both p and q are greater than 0.

Using the definition of the beta density (eq. A.1), we may write

$$\begin{aligned} x \cdot h'(x;p,q) &= x \cdot x^{p-1}(1-x)^{q-1}/B(p,q) \\ &= \frac{B(p+1,q)}{B(p,q)}x^{p}(1-x)^{q-1}/B(p+1,q) \\ &= \frac{B(p+1,q)}{B(p,q)}h'(x;p+1,q) \end{aligned}$$

The average mass $V_t^*(v, v)$ of those fish that will remain in their mass class v, given in eq. 8, can be computed by

$$\begin{aligned} V_t^*(v,v) &= \frac{\int_v^{v'} w \cdot h(w;p,q)\,\mathrm{d}w}{a} \\ &= \frac{\int_0^{v'-v} (x+v)h'(x;p,q)\,\mathrm{d}x}{a} \\ &= \frac{\int_0^{v'-v} xh'(x;p,q)\,\mathrm{d}x}{a} + v\frac{\int_0^{v'-v} h'(x;p,q)\,\mathrm{d}x}{a} \\ &= \frac{B(p+1,q)}{B(p,q)} \cdot \frac{\int_0^{v'-v} h'(x;p+1,q)\,\mathrm{d}x}{a} + v \end{aligned}$$

Here, a is the proportion of fish remaining in the mass class v in month t, defined in eq. 3. To simplify the notation, the indices v and t are here omitted from h, h', and a.

Appendix B. Empirical prediction errors

The model for the standing stock, conditioned on the values of removed and stocked fish and sea temperatures, is essentially deterministic. The reason is that we require a model that is causal in structure and strictly fulfils the principles of balance of numbers and biomass. This is easier to achieve in a deterministic model. However, the data contain measurement errors and the model is not perfect (model error). To take this uncertainty into account when calculating the prediction uncertainty, we calculate empirical residuals. Then we add samples from these empirical residuals to the predicted values.

The empirical k-step-ahead prediction errors are the differences between predicted and observed values

$$\begin{aligned} \tilde{e}^{N}_{t+k} &= \hat{N}_{t+k|t}(v) - N^{\mathrm{obs}}_{t+k}(v) \\ \tilde{e}^{V}_{t+k} &= \hat{V}_{t+k|t}(v) - V^{\mathrm{obs}}_{t+k}(v) \end{aligned}$$

For each time point t, a consecutive series of empirical prediction errors is calculated for $k = 1$ up to usually $k = 24$ for both numbers and mass to keep the correlation structure between prediction errors of numbers and mass and between different prediction horizons. When predicting, we first predict for the entire prediction horizon and then add a sample of the correlated prediction errors afterwards. When predicting 24 months ahead, this sample consists of 24 consecutive prediction errors from one historical 24-month prediction. This is repeated several times, giving a distribution of the prediction errors.

Appendix C. Submodels for number of fish lost, slaughtered, and wasted

When updating the number of fish in each mass class from one time point to the next, the order of the different steps influences the result. The fish removed at each time step is the sum of fish lost, slaughtered, and wasted. First, we update the number of fish lost, then the number of fish slaughtered and wasted, and finally the growth of the fish remaining, where some fish grow into the next mass class. In addition, the stocked fish are added in mass class 0.

Therefore, the number of fish lost at time t in mass class v can at most be the number of fish in that mass class at time $t - 1$. For a given probability $p^{\mathrm{L}}_{t,v}$, the number of fish lost $N^{\mathrm{L}}_t(v)$ is binomially distributed $(p^{\mathrm{L}}_{t,v}, N^{\mathrm{L}}_t(v))$. To allow for overdispersion, we further assume that the probability $p^{\mathrm{L}}_{t,v}$ is stochastic and follows a beta distribution with expectation $\pi^{\mathrm{L}}_{t,v}$ and an additional parameter a^{L}_v, where a^{L}_v corresponds to the parameter p in eq. A.1. The expectation $\pi^{\mathrm{L}}_{t,v}$ depends on the time and the mass class, while a^{L}_v depends on the mass class only. The number of fish lost is therefore beta-binomially distributed (Agresti 2002):

$$\text{(C.1)}\qquad N^{\mathrm{L}}_t(v) \sim \text{beta-binomial}[\pi^{\mathrm{L}}_{t,v}, a^{\mathrm{L}}_v, N_{t-1}(v)]$$

The expected proportion or probability $\pi^{\mathrm{L}}_{t,v}$ is modelled as a smoothly varying trend, using a logit link where the linear predictor is a smooth function of time (Appendix F) plus a seasonal adjustment with one parameter per month.

After fish lost have been removed, there are $N_{t-1}(v) - N_t^{\mathrm{L}}(v)$ left. Let $N_t^{\mathrm{SW}}(v) = N_t^{\mathrm{S}}(v) + N_t^{\mathrm{W}}(v)$ be the sum of slaughtered and wasted fish. Similar to fish lost $(N_t^{\mathrm{L}}(v))$, N_t^{SW} is modelled as a proportion of remaining fish:

$$N_t^{\mathrm{SW}}(v) \sim \text{beta-binomial}[\pi_{t,v}^{\mathrm{SW}}, a_v^{\mathrm{SW}}, N_{t-1}(v) - N_t^{\mathrm{L}}(v)]$$

Reference

Agresti, A. 2002. Categorical data analysis. Wiley, Hoboken, N.J.

Appendix D. Model for mass of slaughtered fish

Monthly data are available for mean gutted mass of slaughtered fish in each mass class, which is lower than the mass of live fish. The gutted mass of slaughtered fish is assumed to be proportional to the mass per fish in the same mass class in the same month and follows a gamma distribution;

$$V_t^{\mathrm{G}}(v) \sim \text{gamma}[\beta_{v,t}^{\mathrm{G}} \cdot V_t(v), a_v^{\mathrm{G}}]$$

where the product $\beta_{v,t}^{\mathrm{G}} \cdot V_t(v)$ is the expected value per month, and a_v^{G} is the shape parameter. In addition, we let the proportionality factor $\beta_{v,t}^{\mathrm{G}}$ vary smoothly with time (Appendix F). This model, as opposed to eq. 12, falls into the generalized linear modelling framework (McCullagh and Nelder 1989).

Reference

McCullagh, P., and Nelder, J.A. 1989. Generalized linear models. 2nd edition. Chapman and Hall, London, UK.

Appendix E. Model for sea temperature

The sea temperature affects the growth. Hence, to predict and simulate future possible growth, we have to predict and simulate the sea temperature. We assumed that the temperature varies randomly around a seasonal term and that the temperature may not deviate too much from this trend, where the deviations (residuals) are modelled as an AR(1) process:

$$\begin{aligned} \mathrm{ST}_t &= A_t + \varepsilon_t \\ \varepsilon_t &= \alpha\varepsilon_{t-1} + w_t \end{aligned}$$

where $w_t \sim N(0, \sigma_{\mathrm{ST}}^2)$. Here, A_t is a seasonal term described by seven parameters:

$$\begin{aligned} A_t = \beta_0^{\mathrm{ST}} &+ \beta_1^{\mathrm{ST}} \sin\left(\frac{2\pi t}{12}\right) + \beta_2^{\mathrm{ST}} \cos\left(\frac{2\pi t}{12}\right) \\ &+ \beta_3^{\mathrm{ST}} \sin\left(\frac{4\pi t}{12}\right) + \beta_4^{\mathrm{ST}} \cos\left(\frac{4\pi t}{12}\right) \\ &+ \beta_5^{\mathrm{ST}} \sin\left(\frac{8\pi t}{12}\right) + \beta_6^{\mathrm{ST}} \cos\left(\frac{8\pi t}{12}\right) \end{aligned}$$

Appendix F. Time trend

In the models for the number of stocked fish, the number of fish lost, slaughtered, and wasted, and mass for slaughtered fish, we use a smooth time trend to handle changes in the overall level over time. Such changes may occur because of improved farming in some sense (fewer deaths) or changed routines, like gradually slaughtering heavier fish. The time trend is modelled by B-splines (Eilers and Marx 1996). A B-spline of degree q consists of $q + 1$ polynomial pieces, each of degree q, on n' intervals. Let $t = 1, 2, \ldots, T$ be the historical times (number of months since the first observation). Then the time level is given by the linear combination

$$\text{(F.1)} \qquad s(t) = \sum_{l=1}^{r} \alpha_l b_l(t)$$

where $b_l(t)$ denotes the lth B-spline with regression coefficient α_l, and r is the number of equidistant B-splines covering the interval $[1,T]$. We chose B-splines for their flexibility, and at the same time they fit in the linear regression modelling framework. A further improvement would have been to include penalties to facilitate further flexibility, while still controlling the effective number of parameters (Eilers and Marx 1996).

When adding extra parameters, as we did for the time trend, we ran the risk of overfitting. Partly by considering Akaike's information criteria (Pawitan 2001), we saw that the time trend was justified, with $q = 2$ and $r = 4$ in eq. F.1, at least for the major mass classes (0–5), which are the important ones. This criterion has also been used for selecting the sea temperature model (Appendix E).

When predicting, we used $s(T)$ for all future time points. Hence, we assumed a constant level for future time points, since we did not assume that we can predict future time level changes.

References

Eilers, P.H.C., and Marx, B.D. 1996. Flexible smoothing with *B*-splines and penalties. Stat. Sci. **11**(2): 89–121. doi:10.1214/ss/1038425655.

Pawitan, Y. 2001. In all likelihood: statistical modelling and inference using likelihood. Oxford University Press, Oxford, UK.

Fine-scale population genetic structure of the yellow perch *Perca flavescens* in Lake Erie

Osvaldo J. Sepulveda-Villet and Carol A. Stepien

Abstract: Discerning the genetic basis underlying fine-scale population structure of exploited native species and its relationship to management units is a critical goal for effective conservation. This study provides the first high-resolution genetic test of fine-scale relationships among spawning groups of the yellow perch *Perca flavescens*. Lake Erie yellow perch stocks comprise valuable sport and commercial fisheries and have fluctuated extensively owing to highly variable annual recruitment patterns. Fifteen nuclear DNA microsatellite loci are analyzed for 569 individuals from 13 primary Lake Erie spawning sites and compared with those spawning in Lakes St. Clair and Ontario. Additional comparisons test for possible genetic differences between sexes and among size–age cohorts. Results demonstrate that yellow perch spawning groups in Lake Erie are genetically distinguishable and do not differ between sexes and among age cohorts. Population genetic structure does not follow a genetic isolation with geographic distance pattern, and some spawning groups contribute more to overall lake-wide genetic diversity than do others. Partitioning of the yellow perch's genetic structure shows little congruence to lake basins or to current management units. Our findings underlie the importance of understanding spawning habitat and behavior to conserve the genetic stock structure of a key fishery.

Résumé : La reconnaissance de la base génétique qui sous-tend la structure de la population à échelle fine chez les espèces indigènes exploitées et de son lien avec les unités de gestion est un objectif essentiel pour une conservation efficace. Notre étude présente le premier test génétique de haute définition des relations à échelle fine entre des groupes de fraie de la perchaude, *Perca flavescens*. Les stocks de perchaudes du lac Érié représentent des pêches sportives et commerciales de grand intérêt et ils ont subi d'importantes fluctuations à cause de leurs patrons annuels de recrutement extrêmement variables. Nous analysons 15 locus microsatellites d'ADN nucléaire chez 569 individus provenant de 13 sites principaux de fraie au lac Érié et les comparons à ceux de poissons qui fraient dans le lac St-Clair et le lac Ontario. Des comparaisons additionnelles vérifient l'existence de différences génétiques possibles entre les sexes et les cohortes de taille–âge. Il est possible de distinguer génétiquement les groupes de fraie de la perchaude dans le lac Érié et il n'y a pas de différence entre les sexes et les cohortes d'âge. La structure génétique de la population ne suit pas un patron d'isolement génétique en fonction de la distance géographique et certains groupes de fraie contribuent plus que d'autres à la diversité génétique globale à l'échelle du lac. Le partitionnement de la structure génétique de la perchaude correspond assez peu aux bassins du lac ou aux unités actuelles de gestion. Nos résultats soulignent l'importance de comprendre les habitats et les comportements de fraie afin de préserver la structure génétique du stock d'une pêche importante.

[Traduit par la Rédaction]

Introduction

Microsatellite DNA markers offer new and effective tools for defining the stock structure of exploited fisheries, important for evaluating population interrelationships (Bernard et al. 2009; Stepien et al. 2009; VanDeHey et al. 2009). Fishery stocks are population subunits that interbreed freely in given geographic locations, share a common gene pool, and differ significantly from other subunits (Hallerman et al. 2003); this genetic-based definition of stock structure is useful for refining effective fishery management units (Waples et al. 2008; Gum et al. 2009; Morin et al. 2010). These genetic stock structure data ideally should be interpreted in light of geographic distributional data throughout the fish's life history, as well as its biological attributes for growth, survival, and recruitment.

The present study uses 15 nuclear DNA microsatellite markers (of higher resolution power than previously analyzed mitochondrial DNA markers) to apply this genetic definition of stock structure towards resolving the fine-scale population relationships of the yellow perch *Perca flavescens* (Teleostei: Percidae) across Lake Erie. Lake Erie is the epicenter of yellow perch sport and commercial fisheries in the Great Lakes (Scott and Crossman 1973; Ryan et al. 2003), with the Lake Erie catch comprising over 4100 metric tonnes (t) in the 2009 season and 4389 t in 2010 (Yellow Perch Task Group (YPTG) of the Lake Erie Committee, Great Lakes Fishery Commission (YPTG 2010, 2011)). Our study addresses the critical goal of the Lake Erie YPTG to discern the genetic basis underlying yellow perch stock structure (Ryan et al. 2003; YPTG 2011).

Received 6 January 2011. Accepted 27 April 2011. Published at www.nrcresearchpress.com/cjfas on 22 August 2011.
J2011-0047

Paper handled by Associate Editor Rolf Vinebrooke.

O.J. Sepulveda-Villet and C.A. Stepien. Great Lakes Genetics Laboratory, Lake Erie Center and Department of Environmental Sciences, The University of Toledo, 6200 Bayshore Road, Toledo, OH 43616, USA.

Corresponding author: Carol A. Stepien (e-mail: carol.stepien@utoledo.edu).

doi:10.1139/F2011-077

Presently, little is known of the number and distribution of yellow perch stocks, the site fidelity of their spawning groups, or of the genetic exchange among them. Previous studies were unable to resolve appreciable fine-scale genetic structure of Lake Erie yellow perch because of lack of resolution power, including mtDNA control region sequences that discerned some differences between eastern basin spawning sites versus others (Ford and Stepien 2004; Sepulveda-Villet et al. 2009). The objectives of the present study are to use nuclear microsatellite data from 15 loci to test for (A) genetic differences among potential Lake Erie stocks, (B) demographic relationships among yellow perch spawning groups, (C) correspondence of genetic data to life history traits, including differences among age cohorts and size classes, and between the sexes, and (D) comparative genetic diversity within and among spawning groups, lake basins, and management units (MUs) used by the YPTG for Lake Erie (of the Great Lakes Fishery Commission), along with their contribution to overall lake-wide levels.

Objective A tests for possible population genetic divergence underlying stock structure based on the alternative–null hypothesis that (1) Lake Erie yellow perch spawning groups are or are not genetically divergent (i.e., they exhibit structure versus panmixia). Objective B examines the demographic relationships among spawning groups to test three alternative–null hypotheses that (2) relationships among spawning groups reflect or do not reflect a pattern of genetic isolation with geographic distance, (3) the spawning groups show or do not show significant genetic demarcations among Lake Erie's three physiographic basins, and (4) the spawning groups correspond or do not correspond to significant genetic demarcations among Lake Erie's four fishery management units. Objective C evaluates the possible influence of life history traits based on the following two hypotheses (alternative–null): (5) male and female yellow perch show or do not show congruent patterns of genetic relationships within and among spawning groups; and (6) age–size cohorts of yellow perch reveal or do not reveal congruent patterns of genetic relationships within and among spawning groups. Objective D assesses relative genetic diversity levels to test the alternative–null hypothesis that (7) the distribution of genetic diversity and its contribution to lake-wide diversity differs significantly or else is comparable among Lake Erie yellow perch spawning groups.

The above relationships are interpreted in light of those supported by our prior study of yellow perch variation using mitochondrial (mt) DNA control region sequences across Lake Erie (Sepulveda-Villet et al. 2009). We also compare present results with the distribution of genetic diversity and divergence in other Lake Erie fishes discerned from microsatellite data, including other percids such as the walleye *Sander vitreus* (Strange and Stepien 2007; Stepien et al. 2009) and the rainbow darter *Etheostoma caeruleum* (Haponski et al. 2009). Recommendations are made for the conservation of yellow perch genetic diversity and stock structure.

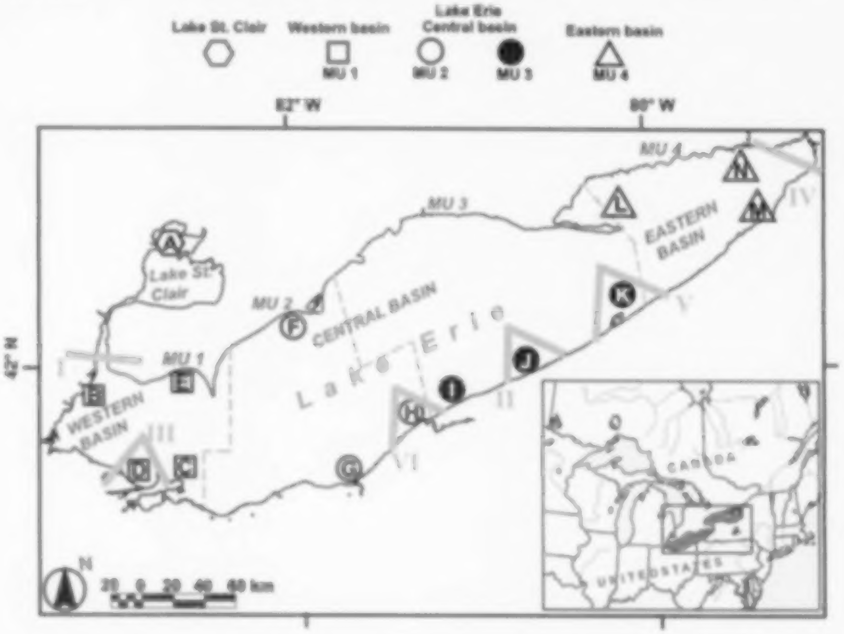
Habitats and life history of Lake Erie yellow perch

It is possible that yellow perch populations may be genetically structured within Lake Erie according to physiographic or bathymetric regions, as prior ecological studies found differences in their distributions according to habitat availability and preference (Wei et al. 2004). Additionally, yellow perch populations may retain the remnants of historic partitioning, reflecting past postglacial colonization routes as discussed by Mandrak and Crossman (1992), with contemporary differences sustained through spawning site philopatry (see Stepien and Faber 1998 and Stepien et al. 2009, 2010 for walleye, and Ford and Stepien 2004 and Sepulveda-Villet et al. 2009 for yellow perch). Kin recognition using olfactory cues has been reported in the European perch *Perca fluviatilis* (Gerlach et al. 2001) and similarly might influence fidelity of yellow perch to spawning sites.

Lake Erie comprises three main bathymetric and physiographic basins (Fig. 1; western, central, and eastern) that are characterized by temperature and depth profiles, each housing differential distributions, habitats, and abundances of yellow perch (Wei et al. 2004; Tyson et al. 2009). The western basin is the shallowest (7.4 m mean depth), warmest (12 °C mean temperature; McCormick and Fahnenstiel 1999), and most turbid (~2 m secchi depth; Bolsenga and Herdendorf 1993) of the three basins and is delimited by Point Pelee and Sandusky Bay following the Pelee–Lorain ridge (Ryan et al. 2003). The western basin is considerably more eutrophic, with greater sedimentation and algal blooms (Tyson et al. 2009), and its shallow islands and reefs contain more yellow perch spawning and nursery habitat than that found to the east (Doremus 1975; Bolsenga and Herdendorf 1993). The central basin (18.5 m mean depth, 10.5 °C mean temperature) is separated from the eastern basin by the Pennsylvania Ridge, and most of its spawning habitat is located along the southern shore (Goodyear et al. 1982). The eastern basin is the deepest (24.4 m mean depth, 64 m maximum depth), coldest (10 °C mean temperature), and most oligotrophic of the three basins (Ryan et al. 2003). Most of its suitable yellow perch habitat is located in Long Point Bay, Ontario, which has abundant spawning and nursery areas (Francis et al. 1985), and along the southern New York shore near Van Buren Bay (Bolsenga and Herdendorf 1993), Sturgeon Point, and Buffalo Knoll (Goodyear et al. 1982).

Male and female yellow perch vary in growth, maturation, and spawning behavior, with females averaging faster growth and reaching larger lengths, masses, and ages (Purchase et al. 2005), and maturing later (3–4 years; Becker 1983) than males (2–3 years; Moyle 2002). Older females usually are more fecund and produce more eggs per spawn, which may be due to their larger sizes rather than age (Schneider 1984). Yellow perch reproductive activity is associated with gradual increase in water temperature during the spring, when large schools of adults congregate in shallow areas of lakes or rivers (Scott and Crossman 1973; Krieger et al. 1983; Jansen et al. 2009). Clusters of two to five males accompany a female during egg deposition and fertilization (Mangan 2004) on submerged vegetation or other benthic structures (Scott and Crossman 1973). Males arrive to the spawning grounds earlier (Scott and Crossman 1973) and tend to linger longer than females, likely fertilizing several females; and neither sex provides parental care (Craig 1987). Genetic structure of spawning groups may differ between males and females owing to differential site fidelity. Our study thus tests for differences in genetic compositions of males versus females within yellow perch spawning groups, as they may display different patterns; this has not been evaluated in other studies.

Fig. 1. Map showing yellow perch spawning sampling sites in Lakes St. Clair and Erie (lettered according to Table 1), with the boundaries of Lake Erie basins and management units (MUs 1–4 as per YPTG 2006). Lines indicate primary barriers to gene flow (ranked I–VI, in order of decreasing magnitude) from the BARRIER analysis (Manni et al. 2004b). Significance of barriers are given as percent bootstrap and number of loci that support it: Barrier I: 75%, 11 loci; Barrier II: 60%, 3 loci; Barrier III: 61%, 5 loci; Barrier IV: 48%, 11 loci; Barrier V: 59%, 6 loci; Barrier VI: 61%, 6 loci.



Yellow perch stocks characteristically display wide variations in annual recruitment that have been attributed to life history characteristics (Beletsky et al. 2007). Historic and current recruitment patterns suggest that size and age trends may impact the overall reproductive success of spawning groups and subsequent recruitment (Henderson and Nepszy 1988). However, possible differences among sizes and age cohorts at given spawning locations have not been addressed in other genetic studies and thus are evaluated here.

Relevance to the yellow perch fishery

The decline of Lake Erie yellow perch recruitment during the 1960s and 1970s and through the mid-1980s was attributed to commercial exploitation, reduction of prey items, and poor water quality (Henderson and Nepszy 1989; Tyson and Knight 2001), preceding a slow recovery through to the present (Munawar et al. 2005; YPTG 2009). During the past decade, Lake Erie yellow perch stocks have increased, yet remain highly variable (Munawar et al. 2005; YPTG 2009). Stock stochasticity underlies yearly management adjustments of Lake Erie's yellow perch total allowable catch (TAC), with the 2006 TAC of 7475 t (YPTG 2006) decreasing to 5165 t in 2007 and 4386 in 2008 (YPTG 2007, 2008) and then increasing to 5448 t in 2009, 5958 t in 2010, and 6404 t in 2011 (YPTG 2009, 2010, 2011). TAC adjustments reflect inherent variability in recruitment from natural and anthropogenic sources.

Yellow perch are not uniformly distributed throughout Lake Erie habitats, and most adults are caught in the central basin during summer months (YPTG 2011). Moreover, yellow perch occupy different habitat regimes than during spawning, as is characteristic across the other Great Lakes (Parker et al. 2009). Although fishery data do not directly reflect fish abundance, trends in spatial fishery catch records suggest major differences in habitat use by perch spawning groups. Most of the total reported Lake Erie yellow perch catch is concentrated in the central basin, totaling 80.5% in 2009 and 75.4% in 2010, whereas the western and eastern basins respectively accrued 15.4% and 4.1% in 2009 and 19.2% and 5.4% in 2010 (YPTG 2010, 2011). Catch also is disproportionate in terms of shorelines; the northern Canadian coast housed ~64% of the 2009 catch and 68% of the 2010 catch, followed by the southern Ohio shorelines that composed ~31% of the total catch and decreased in 2010 to 29%, with the remaining 5% divided by Michigan, Pennsylvania, and New York in 2009, decreasing to only 2.7% in 2010 (YPTG 2010, 2011). The apportionment of catch varies by activity, with most sport fishing occurring in the western basin (46% in 2009, increasing to 47% in 2010), whereas commercial gill nets (allowed in Canada only) and trap nets are predominately used in the central basin (88% for 2009 and 85% for 2010) and the eastern basin (78% in 2009, with 88% in 2010; (YPTG 2010, 2011). Gear type also may differentially affect size and age group compositions of stocks (Kocovsky et al. 2010), which in turn may influence the composition of spawning groups.

The YPTG recognizes four Lake Erie management units (MUs) for yellow perch, which mostly are delineated according to physiographic features or landmarks, including lighthouses and ports (YPTG 2008; see Fig. 1). Our study

Table 1. Sampling locations tested, including sampling year, sample size (N), and mean genetic variability values from 15 microsatellite loci.

Locality	Lat. (°N)	Long. (°W)	N	H_O	H_E	F_{IS}	N_A	R_A	N_{PA}	P_{PA}
Lake St. Clair										
A. Anchor Bay, Mich. (2005)	42.6319	82.77639	39	0.532	0.595	0.106	152	6.799	7	0.046
Lake Erie			516	0.533	0.583	0.085	310	6.856	49	0.158
MU 1 (western basin)			153	0.539	0.583	0.074	250	7.090	15	0.060
B. Monroe, Mich. (2004)	41.8683	83.31778	48	0.559	0.629	0.113	184	7.866	4	0.022
C. Cedar Point, Ohio (2002)	41.4722	82.65833	46	0.521	0.575	0.095	170	7.141	2	0.012
D. South Bass Island, Ohio (2002)	41.6575	83.75361	29	0.551	0.602	0.085	139	7.005	6	0.043
E. Sturgeon Creek, Ont. (2003)	42.0083	82.58750	30	0.525	0.526	0.001	130	6.348	3	0.023
MU 2 (west-central basin)			91	0.555	0.565	0.020	203	6.423	10	0.049
F. Erieau, Ont. (2003)	42.2515	81.91667	30	0.593	0.583	-0.017	146	7.150	1	0.007
G. Cleveland, Ohio (2002)	41.5006	81.82806	41	0.486	0.538	0.097	152	6.654	4	0.026
H. Fairport, Ohio (2003)	41.8058	81.41778	20	0.587	0.575	-0.021	94	5.464	5	0.053
MU 3 (east-central basin)			87	0.519	0.585	0.114	197	6.795	5	0.025
I. Perry, Ohio (2003)	41.8077	81.14522	48	0.522	0.589	0.116	111	7.421	4	0.036
J. Ashtabula, Ohio (2002)	41.9975	80.58972	19	0.533	0.616	0.138	176	6.607	1	0.006
K. Erie, Pa. (2001)	42.1278	80.26944	20	0.503	0.550	0.088	109	6.357	0	0.000
MU 2 + MU 3 (central basin)			178	0.537	0.575	0.067	236	6.609	15	0.064
MU 4 (eastern basin)			185	0.519	0.603	0.139	243	7.180	19	0.078
L. Long Pt. Bay, Ont. (2001)	42.6444	80.22250	60	0.524	0.628	0.166	187	7.624	4	0.021
M. Pt. Colborne – Pt. Albino, Ont. (2001)	42.8444	79.18917	40	0.479	0.597	0.199	165	7.430	5	0.030
N. Dunkirk, N.Y. (2004)	42.5047	79.33389	85	0.554	0.583	0.051	188	6.485	10	0.053
Lake Ontario										
O. Rochester, N.Y. (2002)	43.2880	77.14111	14	0.514	0.616	0.170	100	6.667	4	0.040
Total			569	0.526	0.598	0.120	323	6.774	60	0.186

Note: MU, Lake Erie yellow perch management unit; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; N_A, number of alleles; R_A, allelic richness adjusted by rarefaction; N_{PA}, number of private (unique) alleles per location; P_{PA}, proportion of private alleles per sampling site.

Table 2. Summary statistics for 15 microsatellite loci across the 15 spawning groups of yellow perch from Lakes St. Clair, Erie, and Ontario.

Locus	N_A	Size range (bp)	F_{IS}	F_{IT}	F_{ST}
Svi2	4	210–216	0.081	0.114	0.034
Svi3	12	130–156	0.052	0.108	0.047
Svi4	34	108–182	0.055	0.105	0.037
Svi7	4	176–182	–0.028	0.560	0.555
Svi17	19	142–182	0.341	0.369	0.123
Svi33	41	100–178	0.113	0.139	0.009
YP13	38	231–311	0.100	0.128	0.010
YP17	39	225–311	0.052	0.152	0.120
Mpf1	17	129–169	0.010	0.249	0.260
Mpf2	16	120–160	0.127	0.448	0.321
Mpf3	21	214–280	0.008	0.038	0.021
Mpf4	10	205–224	–0.024	–0.018	0.026
Mpf5	21	107–149	0.145	0.189	0.025
Mpf6	27	173–229	0.018	0.023	0.027
Mpf7	20	148–192	0.081	0.172	0.082
Total	323	—	0.081	0.114	0.034

Note: N_A, number of alleles; bp, base pairs; F_{IS}, average differentiation within a spawning group; F_{IT}, deviation in the total sample; and F_{ST}, average genetic divergence between pairs of spawning groups.

examines whether these MUs are relevant to the genetic stock structure of yellow perch, which may provide insights to management and conservation.

Materials and methods

Sample locations and preparation

State and federal agencies collected adult yellow perch (N = 516 total in Lake Erie) from 13 Lake Erie spawning locations at spawning time (N = 19–85 per site), encompassing its three physiographic basins and four MUs (Fig. 1). Our sampling design was dependent upon the Great Lakes Fishery Commission (GLFC) YPTG's assessment of yellow perch spawning habitat and which sites appeared most relevant to their potential stock structure. Samples additionally were dependent upon the management agencies' sampling regime and resources (e.g., Michigan Department of Natural Resources, Ohio Division of Wildlife, Pennsylvania Fish and Boat Commission, New York Department of Environmental Conservation, Ontario Ministry of Natural Resources, and US Geological Survey). Samples were collected in May–June and were assessed by the agency scientist collectors to be "ripe" or recently "spent". We compared these with representative spawning groups from Lake St. Clair (N = 39) and Lake Ontario (N = 14). Fin clip tissue samples either were preserved in 95% ethanol (EtOH) in the field or immediately frozen. Some perch were tagged and released, while others were sacrificed and used for age and diet studies by Lake Erie fishery managers. They provided us with geographic coordinates (Table 1), as well as available length and sex data. Age and cohort data were determined by the agencies from scale, dorsal spine, or otolith analyses, or by age–length keys.

Microsatellite loci procedure

Genomic DNA was extracted and purified from the tissues with DNeasy Qiaquick kits (QIAGEN, Inc., Valencia, California), and aliquots were frozen and archived. We evaluated 19 microsatellite loci developed for yellow perch and other percid species, including *Svi4*, *Svi11*, *Svi17*, *Svi18*, and *Svi33* from Borer et al. (1999); *Svi2*, *Svi3*, and *Svi7* from Eldridge et al. (2002); *YP6*, *YP13*, *YP16*, and *YP17* from Li et al. (2007); and *Mpf1*–*Mpf7* by Grzybowski et al. (2010). Two loci exhibited evidence of null alleles (*YP6*, *YP16*), and two were monomorphic in Lake Erie (*Svi11*, *Svi18*) and thus were discarded from this study. Our analyses thus were based on 15 loci.

Polymerase chain reactions (PCR) consisted of 50 mmol $KCl{\cdot}L^{-1}$, 1.5 mmol $MgCl_2{\cdot}L^{-1}$, 10 mmol Tris–$HCl{\cdot}L^{-1}$, 50 μmol of each deoxynucleotide$\cdot L^{-1}$, 0.5 μmol each of the forward and reverse primers$\cdot L^{-1}$, 2% dymethyl sulfoxide (DMSO), 5–30 ng DNA template, and 0.6–1.2 μmol *Taq* polymerase$\cdot L^{-1}$ per 10 μL of reaction volume; these were run with negative and positive controls. All reagents and containers were first autoclaved, all surfaces were sterilized with EtOH and ultraviolet radiation, and reactions were performed in a laminar hood. An initial cycle of 2 min at 94 °C for strand denaturation was followed by 40 cycles of denaturation (94 °C, 30 s), primer annealing (1 min) at a primer-specific temperature (Table 2), and polymerase extension (72 °C, 30 s). A final extension at 72 °C for 5 min was included to minimize partial strands.

The forward primers were synthesized with one of four 5′ fluorescent labels, allowing pool-plexing during analysis (grouped as follows: *Svi2* + *Svi7*, *Svi3* + *Svi33*, *Svi4* + *Svi17* + *Svi18*, *YP13* + *YP17*, *Mpf1* + *Mpf2* + *Mpf5* + *Mpf6*, and *Mpf3* + *Mpf4* + *Mpf7*). Amplification products were processed for allele length analysis by diluting at a ratio of 1:50 with dH_2O, then a 1 μL aliquot was added to 13 μL of a formamide and ABI GeneScan-500 size standard solution, loaded onto a 96-well plate, and denatured for 2 min at 95 °C. The denatured products were analyzed on our ABI 3130XL Genetic Analyzer with GENEMAPPER ver. 3.7 software (Applied Biosystems Inc., Foster City, California).

All output profiles were reviewed manually to confirm correct identification of the allelic size variants.

Data analyses

Spawning population samples were tested for conformance to Hardy–Weinberg equilibrium (HWE) expectations at each locus, whose significance was estimated using the Markov chain Monte Carlo method and 1000 randomization procedures (Guo and Thompson 1992) in GENEPOP ver. 4.0 (Rousset 2008). Deviations were tested for both scenarios of heterozygosity deficiency or excess, and loci were analyzed for linkage disequilibrium (LD). Significance levels for HWE and LD tests were adjusted using Bonferroni corrections (Sokal and Rohlf 1995). Tests for possible occurrence of null (nonamplified) alleles were performed with MICRO-CHECKER ver. 2.2.3 (van Oosterhout et al. 2004, 2006). The number of private (unique) alleles (N_{PA}; those occurring only in a single spawning group) was determined using CONVERT ver. 1.31 (Glaubitz 2004). Number of alleles (N_A) and allelic richness (R_A; the number of alleles per locus, independent of sample size, determined by rarefaction according to El Mousadik and Petit 1996) were calculated using FSTAT ver. 2.9.3.2 (Goudet 2002).

To evaluate objective A and hypothesis 1 of whether yellow perch spawning samples were genetically structured, unbiased θ (Weir and Cockerham 1984) and ρ (Michalakis and Excoffier 1996) estimates of F statistics and their associated levels of significance assessed genetic heterogeneity at different scales in FSTAT. Relationships among such recently diverged populations have been shown to be better resolved in models using θ_{ST} (the F_{ST} estimate of Weir and Cockerham 1984), with values differing significantly from zero used to reject the null hypothesis of panmixia (Balloux and Lugon-Moulin 2002). Pairwise comparisons also were conducted using an exact nonparametric procedure with probability estimates from a Markov chain Monte Carlo method in GENEPOP, which does not rely on assumptions of normality, but has less statistical power (see Goudet et al. 1996). In all pairwise tests, sequential Bonferroni adjustments to probability values were used to minimize type I errors (Rice 1989).

Objective B assessed the demographic partitioning of genetic variation among spawning group samples, with hypothesis 2 testing for correspondence of genetic distance ($\theta_{ST}/1 - \theta_{ST}$) to geographic distance (measured as the shortest waterway distances between pairs of spawning sites) and regression significance interpreted using Mantel's (1967) procedure with 1000 permutations in GENEPOP (Rousset 1997). To further examine relationships among sampling sites, Nei's (1972) pairwise genetic distances (D_s) and Cavalli-Sforza and Edwards' (1967) chord distances (D_c) were calculated using GENDIST in PHYLIP ver. 3.69 (Felsenstein 2008) and used to construct neighbor-joining trees (Saitou and Nei 1987). Relative support values for the nodes of the trees were estimated using 1000 bootstrap pseudoreplicates (Felsenstein 1985) in PHYLIP.

The relative magnitude of genetic structure among yellow perch samples (objectives A and B) was investigated further using the computational geometry-based approach in BARRIER ver. 2.2 (Manni et al. 2004*a*, 2004*b*), which identified geographically discontinuous assemblages of sampling sites, independent from a priori knowledge of their relationships. Pairwise θ_{ST} estimates were mapped onto a matrix of sample geographical coordinates (latitude and longitude), and the relative magnitude of each barrier was ranked according to the number of loci that supported it. Barrier support and rankings were evaluated further with a bootstrap analysis of the multilocus θ_{ST} matrix with 2000 iterations in GENELAND ver. 3.1.4 (Guillot et al. 2005*a*, 2005*b*, 2008), based on the R statistical analysis software suite ver. 2.10.1 (R Development Core Team 2009). Genetic barriers supported by bootstrap values higher than 50% were reported.

To further evaluate distinctive populations (objective B, hypotheses 3 and 4), we employed a Bayesian-based clustering algorithm in STRUCTURE ver. 2.3.3 (Pritchard et al. 2000; Pritchard and Wen 2004) to identify groups of individuals with distinctive allelic frequencies, regardless of their true spawning population identity. Correspondence to spawning populations was analyzed by specifying number of groups (K) in independent runs, ranging from $K = 1$ (the null hypothesis of panmixia) to $K = 15$ (the total N of spawning sites sampled). Ten independent runs were implemented per K, with burn-ins of 100 000 and 500 000 replicates. We examined the consistency among runs, comparative probabilities of individuals assigning to one or more groups, log-likelihood and posterior probability values from each run, and their respective grouping patterns. Optimal K scenarios were determined with the posterior probability procedure of Pritchard et al. (2000) and ΔK likelihood evaluations from Evanno et al. (2005).

The Bayesian program GENECLASS2 (Piry et al. 2004) tested self-assignment of individuals to spawning groups, using a simulated population size of 10 000 individuals per site, with a rejection level of 0.01 (Cornuet et al. 1999). We also used GENECLASS2 to test assignments of individuals in Lake Erie proper to basins and to MUs. We then compared the results of the Bayesian GENECLASS2 and STRUCTURE analyses with population relationships derived from the BARRIER and pairwise genetic divergence analyses. Three-dimensional factorial correspondence analysis (3D-FCA; Benzecri 1973) further explored population divisions and relationships in GENETIX ver. 4.05 (Belkhir et al. 1996–2004), which made no a priori assumptions about population relationships.

To more comprehensively test demographic partitioning in objective B and the potential influence of life history on genetic structure in objective C, we evaluated percent variance and its significance using analysis of molecular variance (AMOVA; Excoffier et al. 1992) scenarios in ARLEQUIN ver. 3.5.12 (Excoffier et al. 2005; Excoffier and Lischer 2010). These hierarchical tests compared relative levels of variation among and within groups of spawning samples (basins, hypothesis 3; and MUs, hypothesis 4), spawning sites within the groups, the sexes (hypothesis 5), and age cohorts (hypothesis 6).

Objective D and hypothesis 7 sought to compare the distribution of genetic diversity among spawning groups, for which we employed the program CONTRIB ver. 1.02 (Petit et al. 1998) to estimate the contribution of each sample to the overall levels of genetic diversity (C_T) and allelic richness (C_{RT}) across Lake Erie. Genetic diversity and allelic richness each were subdivided into two components, representing contributions to variation within samples (components C_S and

C_{RS}) and contributions to differentiation from other groups (C_D and C_{RD}). Positive values denoted significant influence of a sample to overall lake-wide diversity (positive C_S or C_{RS} values) or indicated that the sample diverged from others (positive C_D or C_{RD} values). Negative values indicated that the sample had less genetic diversity (negative C_S or C_{RS} values) or that it did not significantly diverge from others (negative C_D or C_{RD} values) (see Wilson et al. 2009).

Results

Genetic variation within spawning groups

Nineteen loci initially were tested in this study for 549 yellow perch from 13 spawning sites in Lake Erie and single sites in Lakes St. Clair and Ontario (Table 1); all loci were unlinked and conformed to HWE expectations after Bonferroni correction (Table 2). However, four of these loci — *YP*6, *YP*16, *Svi*11, and *Svi*18 — showed evidence of null alleles or were monomorphic and were not further analyzed. We thus based this study on analyses of 15 loci. Overall, loci *Svi*7, *Svi*17, *YP*17, *Mpf*1, and *Mpf*2 were the most informative for discerning divergence among the spawning sites sampled, as evidenced by their higher F_{ST} values (Table 2).

Number of alleles per locus ranged from 4 (*Svi*2 and *Svi*7) to 39 (*YP*17), and their overall number was greatest in the eastern basin sites at Dunkirk (site N) and Long Point Bay (L; Tables 1 and 2). Yellow perch spawning at the Monroe (B) site in the western basin and at Perry in the central basin also had high allelic richness. Central basin spawning sites were characterized by lower numbers of alleles, with the least being found at Fairport (H) and Erie (K). Heterozygosity (H_O) was highest in the western basin sites overall (0.539 mean H_O), with individual site values ranging from 0.479 (Pt. Colborne – Pt. Albino; location M in the eastern basin) to 0.593 (Erieau; location F, central basin), and appeared relatively comparable among Lake Erie spawning sites (mean = 0.533). All spawning groups except Erie (K) had private (i.e., unique) alleles (Table 1), which varied in frequency from one (Erieau, site F; and Ashtabula, site J) to 10 (Dunkirk, site N), indicating a higher proportion of private alleles in the eastern basin (Table 1). No significant variations in allele richness, frequencies, or distributions were detected between males or females in the nine sites for which gender data were available.

Divergence among spawning groups

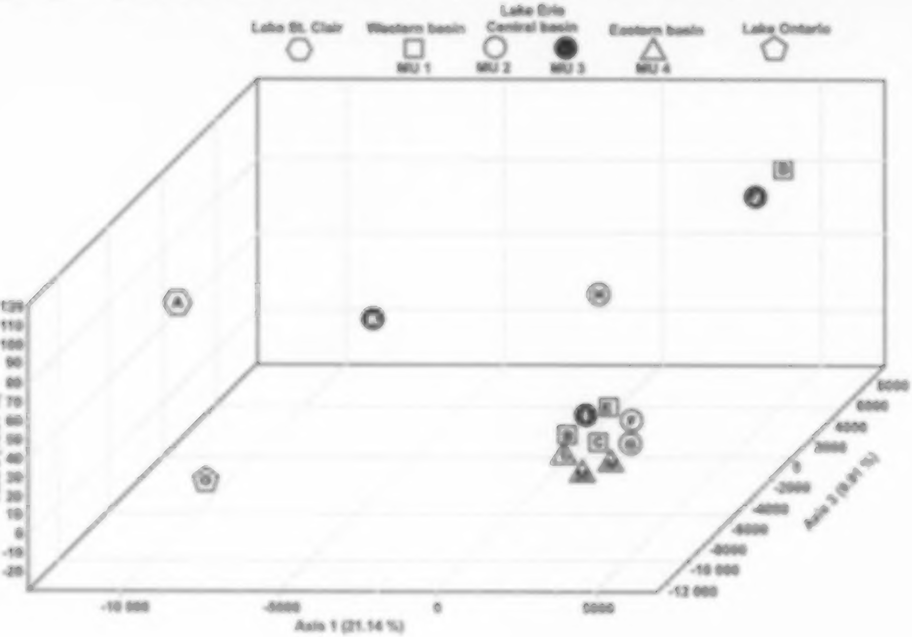
Most yellow perch spawning samples genetically differed from those spawning at other locations, refuting the null hypothesis of panmixia (objective A, hypothesis 1) and supporting the alternative hypothesis of multiple yellow perch stocks in Lake Erie. Almost all pairwise comparisons (171 of 177) remained significantly divergent following sequential Bonferroni correction (Table 3), and results from θ_{ST} and ρ_{ST} comparisons were congruent, showing that almost all spawning sites housed distinct gene pools. The average θ_{ST} between yellow perch in each of the three Lakes was 0.230, whereas the mean difference within the Lake Erie sites was 0.191, ranging from 0.000 to 0.665. Pairwise comparisons between Lake Erie sites that did not significantly differ were Monroe (B) vs. Erieau (F), Cedar Point (C) vs. Long Point Bay (L) and Pt. Colborne – Pt. Albino (M), Ashtabula (J)

Table 3. Summary statistics for pairwise tests of yellow perch population sample heterogeneity using 15 loci.

Basin	MU	Location	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Lake St. Clair	—	A. Anchor Bay, Mich.	—	0.342	0.456	0.461	0.598	0.337	0.481	0.090	0.496	0.476	0.078	0.444	0.441	0.579	0.550
Western Lake Erie	1	B. Monroe, Mich.	0.344	—	0.042	0.037	0.144	*0.002*	0.030	0.361	0.049	0.056	0.353	0.053	0.035	0.142	0.200
		C. Cedar Point, Ohio	0.458	0.042	—	0.076	0.142	0.080	*0.016*	0.473	0.090	0.104	0.479	*0.001*	*0.000*	0.080	0.128
		D. South Bass Isl., Ohio	0.464	0.040	0.078	—	0.118	0.053	0.026	0.503	*0.000*	0.040	0.488	0.102	0.074	0.094	0.285
		E. Sturgeon Creek, Ont.	0.599	0.145	0.142	0.120	—	0.153	0.080	0.666	0.088	0.040	0.632	0.172	0.147	0.065	0.296
Central Lake Erie	2	F. Erieau, Ont.	0.339	*0.003*	0.080	0.055	0.153	—	0.050	0.375	0.055	0.059	0.357	0.101	0.076	0.177	0.254
		G. Cleveland, Ohio	0.483	0.031	*0.016*	0.029	0.080	0.050	—	0.509	0.027	0.045	0.505	0.046	0.022	0.058	0.218
		H. Fairport, Ohio	0.091	0.361	0.473	0.506	0.665	0.374	0.510	—	0.573	0.512	0.113	0.458	0.456	0.594	0.621
	3	I. Perry, Ohio	0.499	0.051	0.091	*0.000*	0.088	0.056	0.030	0.574	—	0.020	0.545	0.110	0.077	0.094	0.304
		J. Ashtabula, Ohio	0.479	0.059	0.107	0.046	0.047	0.062	0.049	0.516	0.028	—	0.473	0.122	0.092	0.106	0.266
		K. Erie, Pa.	0.079	0.354	0.479	0.491	0.632	0.357	0.505	0.110	0.477	0.547	—	0.465	0.460	0.589	0.594
Eastern Lake Erie	4	L. Long Point Bay, Ont.	0.447	0.055	*0.003*	0.106	0.176	0.105	0.048	0.461	0.127	0.115	0.468	—	*0.000*	0.119	0.098
		M. Pt. Colborne – Pt. Albino, Ont.	0.445	0.038	*0.000*	0.079	0.151	0.080	0.025	0.461	0.099	0.084	0.465	*0.000*	—	0.100	0.118
		N. Dunkirk, N.Y.	0.581	0.144	0.082	0.097	0.066	0.179	0.060	0.596	0.109	0.098	0.591	0.122	0.104	—	0.230
Lake Ontario	—	O. Rochester, N.Y.	0.554	0.203	0.130	0.291	0.300	0.256	0.220	0.623	0.275	0.310	0.597	0.106	0.124	0.234	—

Note: Data above diagonal are F_{ST} analog values ρ_{ST} (Michalakis and Excoffier 1996), and data below diagonal are θ_{ST} (Weir and Cockerham 1984). Underlined and italic data are not significant; underlined only data are significant at 0.05 level; all other data also are significant following sequential Bonferroni correction (Rice 1989). All comparison results were congruent with an exact test of differentiation (Goudet et al. 1996).

Fig. 2. Three-dimensional factorial correspondence analysis (Benzecri 1973; GENETIX ver. 4.05, Belkhir et al. 1996–2004) showing relationships among yellow perch spawning groups (labeled according to Table 1) based on 15 microsatellite loci.



vs. South Bass Island (D) and Perry (I), and Long Point Bay (L) vs. Pt. Colborne – Pt. Albino (M). Results from the exact tests were congruent with these findings, indicating that these variations were not influenced by sample size.

Genetic differences among Lake Erie yellow perch spawning groups appeared independent of spatial isolation or geographical distance (objective B, supporting the null hypothesis 2), indicated by the regression of genetic distances (θ_{ST} / 1 – θ_{ST}) versus nearest waterway distances (km; p = 0.212, R^2 = 0.024). Thus, some nearby groups were very divergent (notably Perry, I; Erie, K; and South Bass Island, D), and some distant sites appeared similar (e.g., South Bass Island, D; and Ashtabula, J).

Phylogenetic trees (not shown) based on Nei's genetic distance and Cavalli-Sforza chord distances showed congruent relationships and levels of bootstrap support, with the Erie (K) sample appearing basal from all other Lake Erie sites, and significant genetic distances separating the Fairport (H) spawning group from the others (62.7% support), clustering Long Point Bay (L) with Pt. Colborne – Pt. Albino (M; 78.6% support), and separating South Bass Island (D) and Ashtabula (J) from Erieau (F; 90.1% support). Those were the sole relationships supported by bootstrap values. Similarly, the 3D-FCA (Fig. 2) explained 47.56% of the overall variation, clustering all eastern basin spawning groups (L–N) near some of the samples from the central and western basins (B–C, E–G, and J). However, yellow perch spawning at South Bass Island (D) and Ashtabula (J) clustered separately from this main group, and Fairport (H) and Erie (K) also were separated from all others, showing no clear pattern of structure by basin or MU or geographic proximity (Fig. 2). Thus, all three null hypotheses were supported for objective B, refuting overall correspondence of genetic relationships to geographic distance (hypothesis 2), among the three Lake basins (hypothesis 3), or with the four MUs (hypothesis 4).

AMOVA partitioning tests likewise revealed no significant relationships of yellow perch genetic structure to Lake Erie's three physiographic basins (p = 0.887; objective B, hypothesis 3; Table 4, row *a*), or with the MUs (p = 0.789; hypothesis 4; Table 4, row *b*). The relationship between genetic structure and temporal variation was significant (p = 0.004; Table 4, row *c*), indicating that sampling year had some influence on genetic structure among Lake Erie yellow perch spawning groups, which was less pronounced than the divergence pattern among the sites (p = 0.001). Neither gender distribution (p = 0.443) nor age cohort composition (p = 0.848) had a significant effect on yellow perch genetic structure (objective C, hypotheses 5 and 6; see Table 4, rows *d* and *e*).

Barrier analysis indicated the presence of some genetic discontinuities among Lake Erie spawning groups, with the highest-ranking barrier (barrier I; 75% bootstrap, supported by 11 loci) separating Lake St. Clair from Lakes Erie and Ontario (Fig. 1). Barrier II isolated the spawning group from Ashtabula (J) from all others (60% bootstrap, 3 loci). Barrier III isolated the spawning sample from South Bass Island (D) from all others in Lake Erie (61% bootstrap, 5 loci). Barrier IV distinguished Lake Ontario (O) samples (48% bootstrap, 11 loci), whereas barriers V and VI isolated spawning groups from Erie (K; 59% bootstrap, 6 loci) and Fairport (H; 61% bootstrap, 6 loci), respectively (Fig. 1).

Bayesian STRUCTURE analyses shown in Fig. 3 identified K = 10 population clusters that clearly distinguished yel-

Table 4. Relative distribution of genetic variation among and within yellow perch spawning sites in Lake Erie using analysis of molecular variance (AMOVA).

Source of variation	% variation	Fixation index	P
(*a*) Among the three physiographic basins	1.01	−0.010	*0.887*
Among sampling sites within basins	7.09	0.061	0.001
Within the sampling sites	91.90	0.070	0.001
(*b*) Among management units (MUs) 1–4	1.14	−0.011	*0.789*
Among the sampling sites within MUs	7.26	0.061	0.001
Within the sampling sites	91.60	0.072	0.001
(*c*) Among collection years (2001–2005)	2.34	0.023	0.004
Among sampling sites within years	6.47	0.066	0.001
Within sampling sites	91.18	0.088	0.001
(*d*) Between the sexes	0.17	−0.002	*0.443*
Among sampling sites within sexes	5.54	0.055	0.001
Within sampling sites	94.29	0.054	0.001
(*e*) Among age cohorts	0.95	−0.009	*0.848*
Among sampling sites within age cohorts	4.58	0.045	0.001
Within sampling sites	94.47	0.036	0.001

Note: (*a*) Three Lake Erie basins: western, central, and eastern; (*b*) four Lake Erie management units (MUs); (*c*) four collection years (2001, 2002, 2003, and 2004); (*d*) male and female yellow perch; (*e*) nine age cohorts (1992–2002). Underlined and italicized data are not significant; regular text data are significant at $P \leq 0.05$ level.

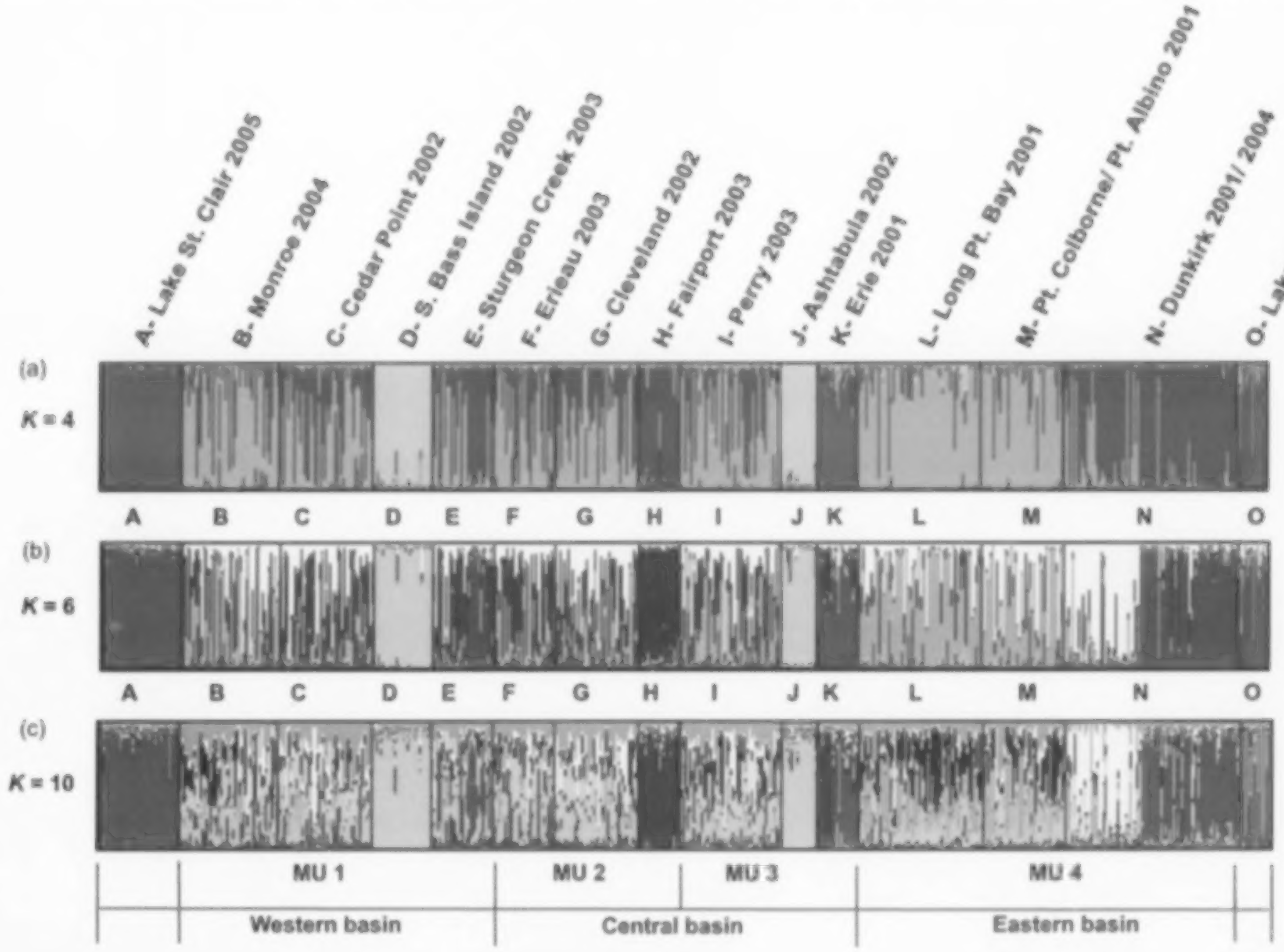
low perch spawning groups from Lakes St. Clair (site A; colored red), Ontario (O; green), and Erie. Within Lake Erie, unique clusters distinguished spawning groups from Fairport (H; teal) and Erie (K; red), and another linked spawning groups from South Bass Island with Ashtabula (D and J; pink). Nearby eastern basin spawning groups from Long Point Bay and Pt. Colborne – Pt. Albino (L and M) shared similar allelic compositions. Samples from Dunkirk (N) appeared very different in 2001 (lime) and 2004 (blue), with the latter appearing more like that from Sturgeon Creek (E; 2003). The $K = 4$ and $K = 6$ scenarios also were supported by the likelihood method (Evanno et al. 2005) and denote the most pronounced differences among the population stocks in Lakes St. Clair, Erie, and Ontario, as well as the apparent similarity between the groups spawning at South Bass Island and Ashtabula (D and J; pink).

Bayesian assignment tests in GENECLASS2 correctly self-assigned 100% of samples to Lakes St. Clair (A) and Ontario (O), respectively (Table 5). No individuals from Lakes St. Clair or Ontario assigned to Lake Erie spawning sites, and only five perch from Erie (K) assigned to Lake St. Clair (A), indicating very little or no gene flow among the three lakes (Table 5*a*). Individual sites from spawning locations in the western and eastern Lake Erie basins overall had the highest self-assignments to individual sites (Table 5*a*), basins (Table 5*b*), and to MUs (Table 5*c*). Individual spawning sites in Lake Erie with strongest self-assignments were South Bass Island (D; 75.9%), Erie (K; 65%), Long Point Bay (L; 61.7%), Fairport (H; 55%), Monroe (B; 54.2%), and Ashtabula (J; 52.6%). Although 54% of fish from Monroe (B) self-assigned, an appreciable proportion of individuals from other spawning sites also assigned to the Monroe site (25.1%), including some of those from Cedar Point (C), Erieau (F), Cleveland (G), and Perry (I; Table 5*a*). Spawning sites in the central basin had the lowest self-assignment values, which also was evident in the overall basin and MU assignment tests (Tables 5*b* and 5*c*). Results from GENECLASS2 analyses (Table 5) overall appeared similar to the clustering assignments from the program STRUCTURE (Fig. 3).

Relative contributions of sampling sites to genetic diversity (C_T; objective D, hypothesis 7) were found to differ significantly in the CONTRIB analyses, supporting the alternative hypothesis, as shown by the different bar heights and values in Fig. 4*a*. Samples with positive differentiation values (C_D; grey bars), in terms of their contribution to overall genetic diversity, significantly differed from all other sites (depicted with grey bars extending above the zero median line). These included the two outgroups (Lake St. Clair (A) and Lake Ontario (O)) and three Lake Erie sites (South Bass Island (D), Ashtabula (J), and Erie (K)). These results are similar to those distinguishing the same sampling sites in other analyses, including F_{ST} comparisons (Table 3), 3D-FCA (Fig. 2), and STRUCTURE (Fig. 3). The other sampling sites did not differ significantly in this overall genetic differentiation component (C_D; grey bars are negative; Fig. 4*a*). Samples with positive black bars (C_S) in Fig. 4*a* contributed to overall lake-wide genetic diversity with unique alleles, including Monroe (B), Long Point Bay (L), Pt. Colborne – Pt. Albino (M), Perry (I), and Dunkirk (N). Other samples had only common widespread alleles (these had negative black C_S bars).

The relative distribution of allelic richness among the sampling sites (C_D) distinguished only a single sampling location (Lake St. Clair, A) in terms of the component of allelic differentiation (C_{RD}; positive grey bar), with two others

Fig. 3. Estimated yellow perch population structure from Bayesian STRUCTURE analyses (Pritchard et al. 2000; Pritchard and Wen 2004) for $K = 4$, 6, and 10 groups using 15 loci. Optimal $K = 10$ (posterior probability (pp) = 0.999) was determined from ΔK likelihood evaluations (Evanno et al. 2005). The strongest relationships also are evident at the lower K values. Thin vertical lines, partitioned into K colored segments that represent the estimated membership fractions, represent individuals. Black lines separate different spawning samples.



having slightly positive values (South Bass Island, D; and Ashtabula, J). Four other sites showed some positive contribution to allelic diversity (C_{RS}; positive black bar): Monroe (B), Long Point Bay (L), Perry (I), and Pt. Colborne – Pt. Albino (M).

Results from both C_T (total diversity across the sampling regime; Table 4, row *a*) and C_{RT} (total allelic richness; Table 4, row *b*) thus indicated that most diversity in Lake Erie was contributed by a single spawning group in the western basin (Monroe, B), one in the central basin (Perry, I), and two in the eastern basin (Long Pt. Bay, L; and Pt. Colborne – Pt. Albino, M), whereas three groups in Lake Erie appear highly differentiated from the others: South Bass Island (D), Ashtabula (J), and Erie (K).

Discussion

Patterns of genetic diversity in Lake Erie spawning groups

Prior studies discerned relatively low genetic diversity in yellow perch (Strittholt et al. 1988; Billington 1993; Todd and Hatcher 1993), which was attributed to exploitation since the early 1900s (Strittholt et al. 1988) or the result of bottlenecks due to population size fluctuations (Marsden and Robillard 2004). However, the Eurasian perch *Perca fluviatilis* also has low genetic diversity (Refseth et al. 1998; Nesbø et al. 1998, 1999) as do species of the closely related congener *Gymnocephalus* (Stepien et al. 1998, 2005); thus, relatively low genetic diversity may be a phylogenetic characteristic of this lineage. In the present study, all yellow perch spawning samples had similar heterozygosities (range = 0.479–0.593, mean for Lake Erie = 0.533), which were lower than those reported for walleye spawning groups in Lake Erie using microsatellites (mean = 0.704, range = 0.660–0.780; Strange and Stepien 2007). In contrast, spawning groups of smallmouth bass *Micropterus dolomieu* in Lake Erie exhibited a broader range of heterozygosity using microsatellites, but had a lower overall mean (mean = 0.469, range = 0.376–0.557; Stepien et al. 2007).

Miller's (2003) genetic diversity study of Lake Michigan yellow perch using six microsatellite loci (five of which also were used in our present study) found a mean number of 9.700 alleles, whereas our mean for Lake Erie was 9.987 with 15 loci. Their mean expected heterozygosity was 0.500 for Lake Michigan, whereas ours was 0.583. Thus, the genetic diversities of Lake Michigan and Lake Erie yellow perch appear comparable.

The occurrence of private alleles (i.e., those uniquely found in single spawning groups) in Lake Erie yellow perch (0–10 private alleles per sampling site, totaling 49 private alleles of 310 alleles lake-wide, and a proportion of 0.158 for 15 loci) in this study is similar to that found by Strange and Stepien (2007) for Lake Erie walleye (1–7 private alleles per spawning site, totaling 24 private alleles of 148 lake-wide, and a proportion of 0.162 for 9 loci). Private allele occurrence in yellow perch and walleye spawning sites across Lake Erie appears greater than that characterizing Lake Erie smallmouth bass in a study by Stepien et al. (2007), which found 0–2 private alleles per spawning site, totaling 3 private alleles of 60 lake-wide, and a proportion of 0.050 for 8 loci. Although most yellow perch spawning sites in our study contained some private alleles, their overall proportional representation was relatively low. Most of the genetic divergences among locations resulted from allele frequency and distribution shifts, rather than the influence of private alleles. The possible importance of private alleles in spawning samples is twofold: they denote divergence from other groups that may be linked to unique and localized adaptations, and they may be used to identify fish from specific spawning samples. However, given the low representation of these private alleles in our study, such possibilities appear limited.

Patterns of genetic divergence in Lake Erie spawning groups

The present study discerns genetic divergence of most spawning yellow perch samples across Lake Erie, resolving significantly greater population structure than was found in past studies using more slowly evolving markers. Our recent study of mtDNA control region sequences found evidence supporting divergence of some yellow perch spawning groups in the eastern basin of Lake Erie alone (Sepulveda-Villet et al. 2009), whereas allozyme analyses only resolved variation among lakes (Strittholt et al. 1988; Todd and Hatcher 1993). Nuclear microsatellite analyses of yellow perch in Lake Michigan (Kapuscinski and Miller 2000; Miller 2003) described some significant divergence between summer-caught samples from Green Bay and surrounding inland lake sites and samples in open waters of Lake Michigan (with 12 of 21 pairwise comparisons (57%) being significant following Bonferroni correction). However, their studies used six loci and ours used 15; thus, their sites that were not divergent may have had less resolution power from fewer loci. Fine-scale divergence also was evident using 10 microsatellite loci in the St. Lawrence River system, where yellow perch spawning groups comprised four distinct genetic clusters along a 310 km-long corridor (Leclerc et al. 2008). Grzybowski et al. (2010) used seven microsatellite loci to discern fine-scale genetic structure between Lake Michigan yellow perch spawning in open water versus those in Green Bay (F_{ST} = 0.126). Parker et al. (2009) used microsatellite data (done by us) to discern no within-lake genetic differences among yellow perch juveniles located in wetlands versus nearshore open water habitats during summer months, but resolved overall differences between populations in Lakes Michigan and Huron. Yellow perch site fidelity thus may only occur during spawning.

It should be noted that although yellow perch in our study were sampled at spawning time, on putative spawning sites, and were assessed by managers to be in spawning condition, little actually is known of their spawning site fidelity or migrations at spawning time (Roger Knight and Jeffrey Tyson, Ohio Division of Wildlife, 305 E. Shoreline Drive, Sandusky, OH 44870, USA, personal communication, 2011). Thus, it is possible that yellow perch may move from site to site following spawning or that some may spawn in multiple locations or even that some may be in the area and not spawn. These scenarios would lead to a signal of genetic homogeneity, which our data analyses did not support, since most of our spawning groups significantly differed. However, one or more of these scenarios may account for the evidence of little divergence between some of our geographically distant sam-

Table 5. Assignment of individuals (in rows) to (*a*) yellow perch spawning samples (in columns), (*b*) Lake Erie physiographic basins, and

(*a*) Individual spawning sites.

Basin	MU		A	B	C	D	E	F
Lake St. Clair	—	A. Anchor Bay, Mich. (39)	**39**	0	0	0	0	0
Western Lake Erie	1	B. Monroe, Mich. (48)	0	**26**	4	0	0	3
		C. Cedar Point, Ohio (46)	0	20	**10**	0	0	0
		D. South Bass Island, Ohio (29)	0	0	0	**22**	0	1
		E. Sturgeon Creek, Ont. (30)	0	5	3	0	**2**	0
Central Lake Erie	2	F. Erieau, Ont. (30)	0	15	0	0	0	**3**
		G. Cleveland, Ohio (41)	0	14	4	0	0	1
		H. Fairport, Ohio (20)	0	9	0	0	0	1
	3	I. Perry, Ohio (48)	0	19	0	0	1	3
		J. Ashtabula, Ohio (19)	0	0	0	9	0	0
		K. Erie, Pa. (20)	5	1	0	0	0	0
Eastern Lake Erie	4	L. Long Point Bay, Ont. (60)	0	8	1	0	0	0
		M. Pt. Colborne – Pt. Albino, Ont. (40)	0	8	2	0	0	0
		N. Dunkirk, N.Y. (85)	0	18	1	0	0	0
Lake Ontario	—	O. Rochester, N.Y. (15)	0	0	0	0	0	0
		Total assigned to group	44	143	25	31	3	12
		% assigned from total (*N* = 569)	7.7	25.1	4.4	5.4	0.5	2.1

(*b*) Physiographic basins.

	Western basin	Central basin	Eastern basin
Western basin	**92**	26	35
	60.1%	17.0%	22.9%
Central basin	72	**71**	32
	40.0%	39.4%	17.8%
Eastern basin	38	14	**132**
	20.7%	7.6%	71.7%

(*c*) Management units.

	MU 1	MU 2	MU 3	MU 4
MU 1	**92**	6	20	35
	60.1%	3.9%	13.1%	22.9%
MU 2	42	**17**	16	18
	45.2%	18.3%	17.2%	19.4%
MU 3	30	5	**40**	14
	34.5%	5.4%	43.2%	16.1%
MU 4	38	1	13	**132**
	20.7%	0.5%	7.1%	71.7%

Note: Assignment tests used a simulated population size of 10 000 individuals per site, with a rejection level of 0.01 (Cornuet et al. 1999). Bold values on number of individuals that were assigned to a given location.

ples, especially if these yellow perch moved from one location to another within the sampling regime.

We discern no significant correspondence between genetic divergence and geographic distances in yellow perch spawning populations across Lake Erie. Likewise, our previous yellow perch mtDNA (Sepulveda-Villet et al. 2009) and nuclear microsatellite analyses of spawning walleye samples across Lake Erie (Strange and Stepien 2007; Stepien et al. 2009, 2010) did not correspond to a genetic isolation by geographic distance pattern. This pattern also did not occur in spawning smallmouth bass across Lake Erie (Stepien et al. 2007). Thus, genetic structure of Lake Erie fish spawning groups likely is driven by their differential genetic compositions rather than by geographic distance between spawning sites. This appears consistent with findings of previous studies in which closely located yellow perch groups yielded divergent genetic signatures in Lake Michigan, with groups spawning in open water differing from those in nearby Green Bay and its fluvial-linked Lake Winnebago (Miller 2003; Grzybowski et al. 2010).

Our results show that several spawning groups display extensive divergence from nearby groups, indicating unique genetic identities. Most of our analyses identify spawning yellow perch from South Bass Island (D) and Ashtabula (J) as not significantly differing from one another, but comprising a distinct genetic cluster. Previous work on smallmouth bass (Stepien et al. 2007) also characterized those from South Bass Island as unique from other sites in Lake Erie, but not significantly divergent from Conneaut, Ohio, samples, suggesting a similar pattern of genetic structure. Yellow perch spawning at the Erie site (K) represent another unique genetic group in our study, reinforcing previous tagging studies (*c*) Lake Erie management units (MU).

G	H	I	J	K	L	M	N	O	% self	% basin	% MU
0	0	0	0	0	0	0	0	0	100.0	—	—
1	0	4	0	0	8	2	0	0	54.2	62.5	62.5
1	0	4	0	0	10	1	0	0	21.7	65.2	65.2
0	0	0	6	0	0	0	0	0	75.9	75.9	75.9
0	0	6	0	0	7	5	2	0	6.7	33.3	33.3
0	0	6	0	0	4	3	0	0	10.0	30.0	10.0
1	0	10	0	0	7	3	1	0	2.4	29.3	4.9
0	**11**	0	0	0	0	0	0	0	55.0	60.0	60.0
0	0	**12**	0	0	9	4	0	0	25.0	31.3	25.0
0	0	0	**10**	0	0	0	0	0	52.6	52.6	52.6
0	0	0	0	**13**	0	1	0	0	65.0	65.0	65.0
0	0	2	0	0	**37**	11	0	0	61.7	80.0	80.0
1	0	1	0	0	16	**12**	0	0	30.0	70.0	70.0
0	0	10	0	0	11	3	**42**	0	49.4	65.9	65.9
0	0	0	0	0	0	0	0	**14**	100.0	—	—
4	11	55	16	13	109	45	45	14	—	—	—
0.7	1.9	9.7	2.8	2.3	19.2	7.9	7.9	2.5	39.2	55.5	51.6

diagonal denote self-assignment values from GENECLASS2 analyses (Piry et al. 2004). Underlined data indicate the highest

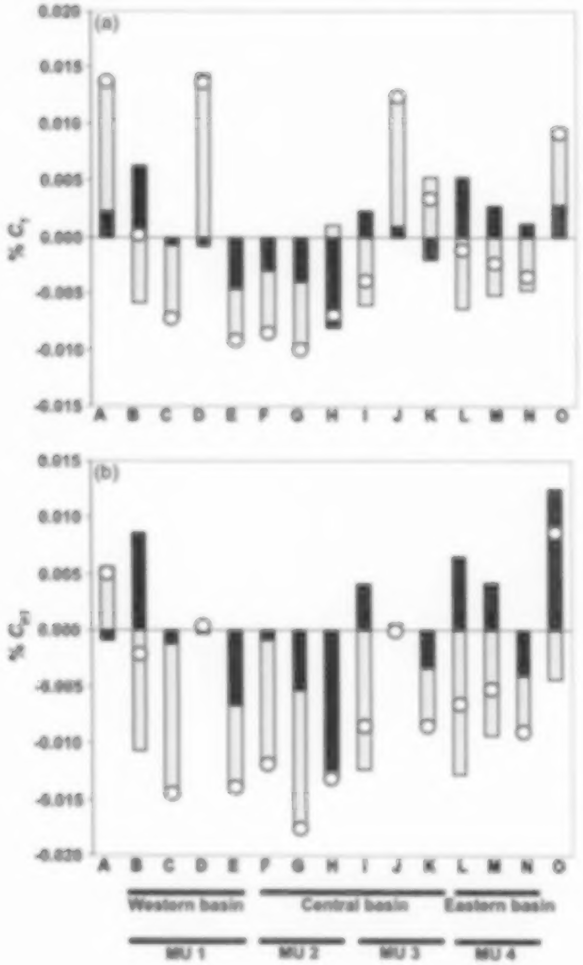
that discerned separate populations from Erie and Presque Isle that differed in growth rates and fecundity from adjacent sampled groups (Kenyon and Murray 2001).

Genetic patterns in relation to other species and fishery management units

Our results indicate moderate yet significant levels of genetic divergence, consistent with patterns in other species (Strange and Stepien 2007; Stepien et al. 2009), showing more pronounced population structure in eastern Lake Erie sites. The distribution of this genetic variation in Lake Erie yellow perch does not correspond to Lake Erie MUs, nor is it significantly associated with physiographic basins. Our previous mtDNA study (Sepulveda-Villet et al. 2009) revealed some genetic divergences among a few yellow perch spawning groups across Lake Erie, including divergence of the eastern basin samples (MU 4), but likewise found no relation to MUs.

Our results indicate that four of the Lake Erie spawning groups had a greater overall contribution to genetic diversity and allelic richness: Monroe (B) in the west basin, Perry (I) in the central basin, and two groups along the northern shore of the eastern basin — Long Point Bay (L) and Pt. Colborne – Pt. Albino (M). The genetic contribution of those spawning groups to Lake Erie yellow perch overall appears high, following diversity and differentiation criteria (see Swatdipong et al. 2009) and thus should continue to be assessed by the GLFC YPTG. Following this strategy, the greatest conservation priority is for rare spawning groups having high levels of both genetic diversity and differentiation (Ashtabula, J), followed by sites with high diversity but lower differentiation (Monroe, B; Perry, I; Long Point

Fig. 4. Contribution of Lake Erie yellow perch samples to total genetic diversity (C_T) and allelic richness (CR_T), as discerned with the program CONTRIB ver. 1.02 (Petit et al. 1998). Spawning groups are labeled as in Table 1. (*a*) Each sample's contribution to total diversity (C_T; circles) is divided into a diversity component (C_S; black bars) and a differentiation component (C_D; grey bars); (*b*) Each sample's contribution to total allelic richness (C_{RT}; circles) is divided into diversity (C_{RS}; black bars) and differentiation (C_{RD}; grey bars) components. Bars with positive values indicate that the spawning sample significantly contributed to overall lake-wide diversity or significantly diverged from other groups. Bars that are negative indicate less genetic diversity or little divergence from other samples.



Bay, L; Pt. Colborne – Pt. Albino, M; and Dunkirk, N), with a third category corresponding to low diversity but high differentiation (South Bass Island, D; Fairport, H; and Erie, K), and then lowest priority for sites having both low diversity and differentiation (Cedar Point, C; Sturgeon Creek, E; Erieau, F; and Cleveland, G). These patterns need to be further assessed for temporal year-to-year variation.

West–east divergence patterns: similarities to other Lake Erie species

We find less genetic diversity (heterozygosity) in spawning groups of yellow perch across Lake Erie than characterized similar studies of walleye spawning populations (Strange and Stepien 2007; Stepien et al. 2009) and more than occurred in smallmouth bass (Stepien et al. 2007). Notably, eastern Lake Erie walleye had greater genetic diversity, whereas eastern basin yellow perch here show comparable heterozygosity levels to those in the western and central basin sites. In contrast, smallmouth bass diversity levels slightly decreased from west to east (0.484–0.452; Stepien et al. 2007). However, patterns of genetic divergence are consistent among the three species in that the eastern basin sites were the most divergent in all studies.

It is possible that this greater divergence of eastern Lake Erie spawning populations from those in the western and central basins reflects their common history of postglacial colonization patterns, as indicated by our previous mtDNA work on yellow perch (Sepulveda-Villet et al. 2009) and other fish species in Lake Erie (Strange and Stepien 2007; Stepien et al. 2009; Haponski and Stepien 2008). Lake Erie perch populations likely originated from Atlantic and Mississippian glacial refugia, whose founding patterns may have been reinforced by adaptations of the eastern basin fish to cooler, deeper, and less productive waters versus those found in warmer, more productive western Lake Erie sites.

Less apparent genetic assignments of central basin yellow perch in our study may reflect a relict signature of population origin, representing a mixture of genotypes originating from the Atlantic refugium to the east and from the Mississippian refugium to the west, as hypothesized for walleye (Stepien and Faber 1998; Strange and Stepien 2007; Stepien et al. 2009). Thus, the apparent lower self-assignments of these central basin yellow perch may represent a relict effect of this historic mixing and not reflect present-day exchange. Therefore, most spawning site samples in our study genetically diverge, yet retain this mixed ancestral signal.

A study by Bergek and Björklund (2007) based on eight microsatellite loci described divergent yet sympatric kin groups of Eurasian perch in a small lake that lacked physical barriers to gene flow. Thus, patterns among Lake Erie yellow perch spawning populations similarly may be maintained by habitat differences and spawning site fidelity. One of the likely barriers to gene flow hypothesized for Eurasian perch is reproductive isolation, either via kin recognition (Gerlach et al. 2001) or because of reduced hybrid fitness between sympatric but divergent cohorts (Behrmann-Godel and Gerlach 2008). These factors remain to be tested for yellow perch.

A recent study by Bergek et al. (2010) suggested that environmental factors other than geographical distance between European perch spawning groups might have led to differences in their genetic composition, with the most likely factor being water temperature differences among habitats during spring spawning, followed by differences in their warming rates. Temperature differences may lead to genetic isolation of various spawning groups, with those in shallow waters spawning earlier (Bergek et al. 2010). These findings highlight the importance of spawning habitat and localized adaptation of their associated reproductive groups. It appears likely that the genetic structure among spawning localities resolved by our study is a product of the interplay between ancestral lineages and environmental variation among spawning areas rather than isolation by distance. This merits further investigation.

Influence of temporal variation on genetic structure of Lake Erie yellow perch

Our AMOVA analyses indicate some variation among collection years in the genetic structure of Lake Erie yellow perch, which is being further tested. However, we found that the overall pattern among spawning locations is much stronger than temporal variation at sites. Franckowiak et al. (2009) evaluated the influence of temporal variation on the effective population size of walleye in Escanaba Lake, Wisconsin, and found that exploitation may have increased genetic drift. The influence of exploitation on the genetic diversity of specific spawning groups of yellow perch in Lake Erie thus should be studied in greater detail.

The "portfolio effect" described by Schindler et al. (2010) hypothesizes that a population's overall ecological stability is dependent upon its baseline genetic composition and diversity range-wide. Consequently, a population that has high genetic diversity (i.e., a larger "portfolio") is more likely to withstand stochastic events or sustained stressors (e.g., exploitation), as opposed to populations with lower genetic diversity (i.e., a lower portfolio). Schindler et al. (2010) used historical data from the sockeye salmon *Onchorhynchus nerka* to discern that temporal variability in a single homogeneous population negatively impacted the fishery via lower recruitment. In contrast, stable levels of salmon returns characterized a scenario in which discrete heterogeneous populations contributed to a temporally stable fishery. Thus, the combination of year-to-year recruitment stochasticity and exploitation on the genetic composition of yellow perch spawning groups may differentially impact their relative contributions to the overall diversity across Lake Erie, which should be tested in further studies. Given the presence of overlapping spawning year classes and cyclical recruitment patterns, as well as the moderate genetic diversity present in the samples we studied, a moderate portfolio effect appears to characterize Lake Erie yellow perch. Thus, anthropogenic stressors could negatively impact the stability of Lake Erie yellow perch stocks, and effective management practices may help to maintain their genetic portfolio.

In conclusion, our investigation demonstrates that most spawning groups of yellow perch are genetically divergent across Lake Erie, with microsatellite loci resolving greater genetic differences than were found using other techniques. Relationship patterns among Lake Erie yellow perch spawning groups likely reflect the interplay between site philopatry, habitat characteristics, and historical glacial origins. We found little evidence for correspondence of Lake Erie yellow perch spawning groups to MUs. Most partitioning of genetic structure among physiographic basins was due to individual spawning site divergence rather than the physical distance among locations or potential environmental barriers to dispersal, such as basin ridges or peninsulas. Differences among groups may reflect habitat partitioning, as has been found for European perch, but remains to be tested for yellow perch. It is unknown whether yellow perch spawning groups retain similar genetic compositions from year to year and whether these relationships among sites are temporally and spatially consistent, which should be investigated. These genetic results should be further coupled with geographic distribution studies throughout the life history of yellow perch towards an integrative approach for defining stock structure.

Acknowledgements

This is publication No. 2011-007 from the Lake Erie Research Center. Grant awards from the NOAA Ohio Sea Grant Program R/LR-7 and R/LR-13, USEPA CR-83281401-0, Lake Erie Protection Fund No. 00-15, USDA NIFA OHOW-2008-03256, and USDA ARS 3655-31000-020-00D supported this study. OJSV received a scholarship award from the International Association for Great Lakes Research and was supported by NSF Gk-12 DGE#0742395 fellowship "Graduate Fellows in STEM High School Education: An Environmental Science Learning Community at the Land–Lake Ecosystem Interface", as well as research and teaching assistantships. We thank P. Allen, D. Clapp, G. Edmond, D. Einhouse, H. Ferris, R. Haas, T. Hartman, K. Kayle, R. Kenyon, C. Knight, R. Knight, P. Kocovsky, A. Naffziger, W. Pearsall, E. Roseman, F. Schram, C. Skelton, M. Thomas, J. Tyson, C. Vandergoot, and C. Yoder, as well as Ohio Department of Natural Resources, Michigan Department of Natural Resources, Pennsylvania Fish and Boat Commission, New York Department of Environmental Conservation, Ontario Ministry of Natural Resources for obtaining specimens, and the US Geological Survey. In addition, we greatly appreciate the advice and help of the Great Lakes Fishery Commission's Lake Erie Yellow Perch Task Group. We especially thank members of our Great Lakes Genetics Laboratory, including J. Banda, J. Brown, A. Haponski, R. Lohner, D. Murphy, M. Neilson, L. Pierce, R. Strange, and T. Sullivan for help. P. Uzmann and M. Gray provided logistic support.

References

Balloux, F., and Lugon-Moulin, N. 2002. The estimation of population differentiation with microsatellite markers. Mol. Ecol. **11**(2): 155–165. doi:10.1046/j.0962-1083.2001.01436.x.

Becker, G.C. 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison, Wisc.

Behrmann-Godel, J., and Gerlach, G. 2008. First evidence for postzygotic reproductive isolation between two populations of Eurasian perch (*Perca fluviatilis* L.) within Lake Constance. Front. Zool. **5**: 3. doi:10.1186/1742-9994-5-3.

Beletsky, D., Mason, D., Schwab, D.J., Rutherford, E., Janssen, J., Clapp, D., and Dettmers, J. 2007. Biophysical model of larval yellow perch advection and settlement in Lake Michigan. J. Great Lakes Res. **33**(4): 842–866. doi:10.3394/0380-1330(2007)33[842:BMOLYP]2.0.CO;2.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. 1996–2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, Université de Montpellier II, Montpellier. Available from http://www.genetix.univ-montp2.fr/genetix/genetix.htm [accessed 20 July 2011].

Benzecri, J.P. 1973. L'analyse des données, II: L'analyse des correspondances. Dunod, Paris.

Bergek, S., and Björklund, M. 2007. Cryptic barriers to dispersal within a lake allow genetic differentiation of Eurasian perch. Evolution, **61**(8): 2035–2041. doi:10.1111/j.1558-5646.2007.00163.x.

Bergek, S., Sundblad, G.S., and Björklund, M. 2010. Population differentiation in perch *Perca fluviatilis*: environmental effects on gene flow? J. Fish Biol. **76**(5): 1159–1172. doi:10.1111/j.1095-8649.2010.02565.x.

Bernard, A., Ferguson, M., Noakes, D., Morrison, B., and Wilson, C. 2009. How different is different? Defining management and conservation units for a problematic exploited species. Can. J. Fish. Aquat. Sci. **66**(9): 1617–1630. doi:10.1139/F09-106.

Billington, N. 1993. Genetic variation in Lake Erie yellow perch (*Perca flavescens*) demonstrated by mitochondrial DNA analysis. J. Fish Biol. **43**(6): 941–943.

Bolsenga, S.J., and Herdendorf, C.E. 1993. Lake Erie and Lake St. Clair handbook. Wayne State University Press, Detroit, Mich.

Borer, S., Miller, L.M., and Kapuscinski, A.R. 1999. Microsatellites in walleye *Stizostedion vitreum*. Mol. Ecol. **8**(2): 336–338.

Cavalli-Sforza, L.L., and Edwards, A.W.F. 1967. Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Genet. **19**(3): 233–257.

Cornuet, J.M., Piry, S., Luikart, G., Estoup, A., and Solignac, M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics, **153**(4): 1989–2000. Available from http://www.genetics.org/content/153/4/1989.full [accessed 20 July 2011].

Craig, J. 1987. The biology of perch and related fishes. Timber Press, Portland, Ore.

Doremus, S.D. 1975. Comments on draft environmental statement. Final environmental statement related to construction of Davis-Besse Nuclear Power Station Units 2 and 3. US Nucl. Reg. Comm. Docket Nos. 50-500 and 50-501. p. A-13.

El Mousadik, A., and Petit, R.J. 1996. Chloroplast DNA phylogeography of the aragan tree of Morocco. Mol. Ecol. **5**(4): 547–555. doi:10.1046/j.1365-294X.1996.00123.x.

Eldridge, W.H., Bacigalupi, M.D., Adelman, I.R., Miller, L.M., and Kapuscinski, A.R. 2002. Determination of relative survival of two stocked walleye populations and resident natural-origin fish by microsatellite DNA parentage assignment. Can. J. Fish. Aquat. Sci. **59**(2): 282–290. doi:10.1139/f02-007.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. **14**(8): 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x.

Excoffier, L., and Lischer, H.E.L. 2010. ARLEQUIN suite. Version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. **10**(3): 564–567. doi:10.1111/j.1755-0998.2010.02847.x.

Excoffier, L., Smouse, P., and Quattro, J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, **131**(4): 479–491. Available from http://www.genetics.org/content/131/2/479.abstract [accessed 20 July 2011].

Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis [online]. Evol. Bioinf. **1**: 47–50. Available from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2658868/ [accessed 20 July 2011].

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**(4): 783–791. Available from http://www.jstor.org/pss/2408678 [accessed 20 July 2011]. doi:10.2307/2408678.

Felsenstein, J. 2008. PHYLIP (Phylogeny Interference Package). Version 3.68. Distributed by the author, University of Washington, Seattle, Wash. Available from http://evolution.genetics.washington.edu/phylip.html [accessed 20 July 2011].

Ford, A.M., and Stepien, C.A. 2004. Genetic variation and spawning population structure in Lake Erie yellow perch, *Perca flavescens*: a comparison with a Maine population. *In* Proceedings of Percis III, the 3rd International Symposium on Percid Fishes. *Edited by* T.P. Barry and J.A. Malison. University of Wisconsin Sea Grant Institute, Madison, Wisc. pp. 131–2.

Francis, G.R., Lino Grima, A.P., Regier, H.A., and Whillans, T.H. 1985. A prospectus for the management of the Long Point ecosystem. Great Lakes Fishery Commission, Ann Arbor, Mich. Tech. Rep. 43. Available from http://www.glfc.org/pubs/TechReports/Tr43.pdf [accessed 20 July 2011].

Franckowiak, R.P., Sloss, B.L., Bozek, M.A., and Newman, S.P. 2009. Temporal effective size estimates of a managed walleye *Sander vitreus* population and implications for genetic-based management. J. Fish Biol. **74**(5): 1086–1103. doi:10.1111/j.1095-8649.2008.02170.x.

Gerlach, G., Schardt, U., Eckmann, R., and Meyer, A. 2001. Kin-structured subpopulations in Eurasian perch (*Perca fluviatilis* L.). Heredity, **86**(2): 213–221. doi:10.1046/j.1365-2540.2001.00825.x.

Glaubitz, J.C. 2004. CONVERT: a user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol. Ecol. Notes, **4**(2): 309–310. doi:10.1111/j.1471-8286.2004.00597.x.

Goodyear, C.S., Edsall, T.A., Dempsey, D.M., Moss, G.D., and Polanski, P.E. 1982. Atlas of the spawning and nursery areas of Great Lakes Fishes. Vol. IX: Lake Erie. US Fish and Wildlife Service, Washington, D.C. Available from http://www.glsc.usgs.gov/_files/atlas/volumes/volume09.pdf [accessed 20 July 2011].

Goudet, J., Raymond, M., de Meeus, T., and Rousset, F. 1996. Testing differentiation in diploid populations. Genetics, **144**(4): 1933–1940. Available from http://www.genetics.org/content/144/4/1933.abstract [accessed 20 July 2011].

Goudet, J. 2002. FSTAT, a program to estimate and test gene diversities and fixation indices, Ver. 2.9.3.2. Available from http://www2.unil.ch/popgen/softwares/fstat.htm [accessed 20 July 2011].

Grzybowski, M., Sepulveda-Villet, O.J., Stepien, C.A., Rosauer, D., Binkowski, F., Klaper, R., Shepherd, B.S., and Goetz, F. 2010. Genetic variation of 17 wild yellow perch populations from the Midwest and east coast analyzed via microsatellites. Trans. Am. Fish. Soc. **139**(1): 270–287. doi:10.1577/T07-276.1.

Guillot, G., Estoup, A., Mortier, F., and Cosson, J.F. 2005*a*. A spatial statistical model for landscape genetics. Genetics, **170**(3): 1261–1280. doi:10.1534/genetics.104.033803.

Guillot, G., Mortier, F., and Estoup, A. 2005*b*. GENELAND: a computer package for landscape genetics. Mol. Ecol. Notes, **5**(3): 712–715. doi:10.1111/j.1471-8286.2005.01031.x.

Guillot, G., Santos, F., and Estoup, A. 2008. Analyzing georeferenced population genetics data with GENELAND: a new algorithm to deal with null alleles and a friendly graphical user interface. Bioinformatics, **24**(11): 1406–1407. doi:10.1093/bioinformatics/btn136.

Gum, B., Gross, R., and Geist, J. 2009. Conservation genetics and management implications for European grayling, *Thymallus thymallus*: synthesis of phylogeography and population genetics. Fish. Manag. Ecol. **16**(1): 37–51. doi:10.1111/j.1365-2400.2008.00641.x.

Guo, S.W., and Thompson, E.A. 1992. Performing the exact test of Hardy–Weinberg proportions for multiple alleles. Biometrics, **48**(2): 361–372. doi:10.2307/2532296.

Hallerman, E., Brown, B., and Epifano, J. 2003. An overview of classical and molecular genetics. *In* Population genetics: principles and applications for fisheries scientists. *Edited by* E.M. Hallerman. American Fisheries Society, Bethesda, Md. pp. 3–20.

Haponski, A.E., and Stepien, C.A. 2008. Molecular, morphological, and biogeographic resolution of cryptic taxa in the Greenside

Darter *Etheostoma blennioides* complex. Mol. Phylogenet. Evol. **49**(1): 69–83. doi:10.1016/j.ympev.2008.07.013.

Haponski, A.E., Bollin, T.L., Jedlicka, M.A., and Stepien, C.A. 2009. Landscape genetic patterns of the rainbow darter *Etheostoma caeruleum*: a catchment analysis of mitochondrial DNA sequences and nuclear microsatellites. J. Fish Biol. **75**(9): 2244–2268. doi:10.1111/j.1095-8649.2009.02414.x.

Henderson, B.A., and Nepszy, S.J. 1988. Recruitment of yellow perch (*Perca flavescens*) affected by stock size and water temperature in Lakes Erie and St. Clair, 1965–85. J. Great Lakes Res. **14**(2): 205–215. doi:10.1016/S0380-1330(88)71549-X.

Henderson, B.A., and Nepszy, S.J. 1989. Factors affecting recruitment and mortality rates of rainbow smelt (*Osmerus mordax*) in Lake Erie, 1963–85. J. Great Lakes Res. **15**(2): 357–366. doi:10.1016/S0380-1330(89)71488-X.

Jansen, A.C., Graeb, B.D.S., and Willis, D.W. 2009. Effect of a simulated cold-front on hatching success of yellow perch eggs. J. Freshwat. Ecol. **24**(4): 651–655. doi:10.1080/02705060.2009.9664343.

Kapuscinski, A., and Miller, L. 2000. DNA-based markers for the assessment of genetic population structure in yellow perch, Project Completion Report to the Great Lakes Fishery Commission, St. Paul, Minn. Available from http://www.fws.gov/midwest/Fisheries/2-Assessmentofgeneticpopulationstructureinyellowperch/Assessment_of_genetic_population_structure_in_yellow_perch.pdf [accessed 20 July 2011].

Kenyon, R., and Murray, C. 2001. A review of the Lake Erie and Presque Isle Bay yellow perch sport angling fisheries, 1997–2001. Pennsylvania Fish and Boat Commission, Lake Erie Research Unit, Fairview, Pa. Available from http://www.fish.state.pa.us/images/fisheries/afm/2001/afm1_leru_01.htm [accessed 20 July 2011].

Kocovsky, P.M., Stapanian, C.T., and Knight, C.T. 2010. Night sampling improves indices used for management of yellow perch in Lake Erie. Fish. Manag. Ecol. **17**(1): 10–18. doi:10.1111/j.1365-2400.2009.00721.x.

Krieger, D.A., Terrell, J.W., and Nelson, P.C. 1983. Habitat suitability information: Yellow perch. US Fish Wildlife Service, Washington, D.C. Available from http://www.nwrc.usgs.gov/wdb/pub/hsi/hsi-055.pdf [accessed 20 July 2011].

Leclerc, E., Mailhot, Y., Mingelbier, M., and Bernatchez, L. 2008. The landscape genetics of yellow perch (*Perca flavescens*) in a large fluvial ecosystem. Mol. Ecol. **27**(7): 1702–1717. doi:10.1111/j.1365-294X.2008.03710.x.

Li, L., Wang, H.P., Givens, C.B., Czesny, S., and Brown, B.L. 2007. Isolation and characterization of microsatellites in the yellow perch (*Perca flavescens*). Mol. Ecol. Notes, **7**(4): 600–603. doi:10.1111/j.1471-8286.2006.01645.x.

Mandrak, N.E., and Crossman, E.J. 1992. Postglacial dispersal of freshwater fishes into Ontario. Can. J. Zool. **70**(11): 2247–2259. doi:10.1139/z92-302.

Mangan, M.T. 2004. Yellow perch production and harvest strategies for semipermanent wetlands in eastern South Dakota. M.Sc. thesis, Wildlife and Fisheries Sciences, South Dakota State University. Available from http://www3.sdstate.edu/wfs/publications/thesis/upload/Mangan-Matthew-T-MS-2004.pdf [accessed 20 July 2011].

Manni, F.E., Guerard, E., and Heyer, E. 2004*a*. Geographical patterns of (genetic, morphologic, linguistic) variation: How barriers can be detected by "Monmonier's algorithm". Hum. Biol. **76**(2): 173–190. doi:10.1353/hub.2004.0034.

Manni, F.E., Guerard, E., and Heyer, E. 2004*b*. Barrier v2.2. Museum of Mankind, Paris. Available from http://www.mnhn.fr/mnhn/ecoanthropologie/software/barrier.html [accessed 20 July 2011].

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. **27**(2): 209–220.

Marsden, J.E., and Robillard, S.R. 2004. Decline of yellow perch in Southwestern Lake Michigan 1987–1997. N. Am. J. Fish. Manage. **24**(3): 952–966. doi:10.1577/M02-195.1.

McCormick, M.J., and Fahnenstiel, G.L. 1999. Recent climatic trends in nearshore water temperatures in the St. Lawrence Great Lakes. Limnol. Oceanogr. **44**(3): 530–540. doi:10.4319/lo.1999.44.3.0530.

Michalakis, Y., and Excoffier, L. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics, **142**(3): 1061–1064. Available from http://www.genetics.org/content/142/3/1061.full.pdf+html [accessed 20 July 2011].

Miller, L.M. 2003. Microsatellite DNA loci reveal genetic structure of yellow perch in Lake Michigan. Trans. Am. Fish. Soc. **132**(3): 503–513.

Morin, P., Martien, K., Archer, F., Cipriano, F., Steel, D., Jackson, J., and Taylor, B.L. 2010. Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. J. Hered. **101**(1): 1–10. doi:10.1093/jhered/esp107.

Moyle, P.B. 2002. Inland fishes of California. University of California Press, Los Angeles, Calif.

Munawar, M., Munawar, I.F., Mandrak, N.E., Fitzpatrick, M., Dermott, R., and Leach, J. 2005. An overview of the impact of non-indigenous species on the food web integrity of North American Great Lakes: Lake Erie example. Aquat. Ecosyst. Health Manage. **8**(4): 375–395. doi:10.1080/14634980500411606.

Nei, M. 1972. Genetic distance between populations. Am. Nat. **106**(949): 283–292. doi:10.1086/282771.

Nesbø, C.L., Magnhagen, C., and Jakobsen, K.S. 1998. Genetic differentiation among stationary and anadromous perch (*Perca fluviatilis*) in the Baltic Sea. Hereditas, **129**(3): 241–249. doi:10.1111/j.1601-5223.1998.00241.x.

Nesbø, C.L., Fossheim, T., Vollestad, L.A., and Jakobsen, K.S. 1999. Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization. Mol. Ecol. **8**(9): 1387–1404. doi:10.1046/j.1365-294x.1999.00699.x.

Parker, A.D., Stepien, C.A., Sepulveda-Villet, O.J., Ruehl, C.B., and Uzarski, D.G. 2009. The interplay of morphology, ecological habitat, resource use, and population genetics in young yellow perch. Trans. Am. Fish. Soc. **138**(4): 899–914.

Petit, R.J., El Mousadik, A., and Pons, O. 1998. Identifying populations for conservation on the basis of genetic markers. Conserv. Biol. **12**(4): 844–855. doi:10.1046/j.1523-1739.1998.96489.x.

Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L., and Estoup, A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. J. Hered. **95**(6): 536–539. doi:10.1093/jhered/esh074.

Pritchard, J.K., and Wen, W. 2004. Documentation for *structure* software: Version 2.2. Department of Human Genetics, University of Chicago, Chicago, Ill. Available from http://pritch.bsd.uchicago.edu/software/structure22/readme.pdf [accessed 20 July 2011].

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, **155**(4): 945–959. Available from http://www.genetics.org/content/164/4/1567.abstract [accessed 20 July 2011].

Purchase, C.F., Collins, N.C., Morgan, G.E., and Shuter, B.J. 2005. Sex-specific covariation among life-history traits of yellow perch (*Perca flavescens*). Evol. Ecol. Res. **7**: 549–566. Available from http://www.freewebs.com/craigpurchase/Purchase_EER.pdf [accessed 20 July 2011].

R Development Core Team. 2009. R: a language and environment for statistical computing r foundation for statistical computing, Vienna. Available from http://www.Rproject.org [accessed 20 July 2011].

Refseth, U.H., Nesbø, C.L., Stacy, J.E., Vollestad, L.A., Fjeld, E., and Jakobsen, K.S. 1998. Genetic evidence for different migration routes of freshwater fish into Norway revealed by analysis of current perch (*Perca fluviatilis*) populations in Scandinavia. Mol. Ecol. **7**(8): 1015–1027. doi:10.1046/j.1365-294x.1998.00423.x.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**(1): 223–225. doi:10.2307/2409177.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics, **145**(4): 1219–1228. Available from http://www.genetics.org/content/145/4/1219.full.pdf+html [accessed 20 July 2011].

Rousset, F. 2008. GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Res. **8**(1): 103–106. Available from http://kimura.univ-montp2.fr/~rousset/Genepop.htm [accessed 20 July 2011].

Ryan, P.A., Knight, R., MacGregor, R., Towns, G., Hoopes, R., and Culligan, W. 2003. Fish community goals and objectives for Lake Erie. Great Lakes Fishery Commission, Windsor, Ont. Spec. Publ. 03-02. Available from http://www.glfc.org/pubs/SpecialPubs/Sp03_2.pdf [accessed 20 July 2011].

Saitou, N., and Nei, M. 1987. The neighbor joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**(4): 406–425 http://mbe.oxfordjournals.org/cgi/reprint/4/4/406.pdf [accessed 20 July 2011].

Schindler, D.E., Hilborn, R., Chasco, B., Boatright, C.P., Quinn, T.P., Rogers, L.A., and Webster, M.S. 2010. Population diversity and the portfolio effect in an exploited species. Nature, **465**(7298): 609–612. doi:10.1038/nature09060.

Schneider, J.C. 1984. Yellow perch maturity and fecundity as a function of age and growth. Fisheries Division, Fisheries Research, Michigan Department of Natural Resources, Lansing, Mich. Rep. No. 1915. Available from http://www.dnr.state.mi.us/publications/pdfs/ifr/ifrlibra/Research/abstracts/1915abs.htm [accessed 20 July 2011].

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Sepulveda-Villet, O.J., Ford, A.M., Williams, J.D., and Stepien, C.A. 2009. Population genetic diversity and phylogeographic divergence patterns of the yellow perch (*Perca flavescens*). J. Great Lakes Res. **35**(1): 107–119. doi:10.1016/j.jglr.2008.11.009.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry, 3rd ed. W.H. Freeman and Co., Inc., New York.

Stepien, C.A., and Faber, J.E. 1998. Population genetic structure, phylogeography and spawning philopatry in walleye (*Stizostedion vitreum*) from mitochondrial DNA control region sequences. Mol. Ecol. **7**(12): 1757–1769. doi:10.1046/j.1365-294x.1998.00512.x.

Stepien, C.A., Dillon, A.K., and Chandler, M.D. 1998. Genetic identity, phylogeography, and systematics of ruffe *Gymnocephalus* in the North American Great Lakes and Eurasia. J. Great Lakes Res. **24**(2): 361–378. doi:10.1016/S0380-1330(98)70827-5.

Stepien, C.A., Brown, J.E., Neilson, M.E., and Tumeo, M.A. 2005. Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: insights for risk analysis. Risk Anal. **25**(4): 1043–1060. doi:10.1111/j.1539-6924.2005.00655.x.

Stepien, C.A., Murphy, D.J., and Strange, R.M. 2007. Broad- to fine-scale population genetic patterning in the smallmouth bass *Micropterus dolomieu* across the Laurentian Great Lakes and beyond: an interplay of behavior and geography. Mol. Ecol. **16**(8): 1605–1624. doi:10.1111/j.1365-294X.2006.03168.x.

Stepien, C.A., Murphy, D.J., Lohner, R.N., Sepulveda-Villet, O.J., and Haponski, A.E. 2009. Signatures of vicariance, postglacial dispersal and spawning philopatry: population genetics of the walleye *Sander vitreus*. Mol. Ecol. **18**(16): 3411–3428. doi:10.1111/j.1365-294X.2009.04291.x.

Stepien, C.A., Murphy, D.J., Lohner, R.N., Haponski, A.E., and Sepulveda-Villet, O.J. 2010. Status and delineation of walleye genetic stock structure across the Great Lakes. *In* Status of Walleye in the Great Lakes: Proceedings of the 2006 Symposium. *Edited by* E. Roseman, P. Kocovsky, and C. Vandergoot. Great Lakes Fishery Commission Technical Report, Ann Arbor, Mich. pp. 189–223.

Strange, R.M., and Stepien, C.A. 2007. Genetic divergence and connectivity among river and reef spawning groups of walleye (*Sander vitreus vitreus*) in Lake Erie. Can. J. Fish. Aquat. Sci. **64**(3): 437–448. doi:10.1139/f07-022.

Strittholt, J.R., Guttman, S.I., and Wissing, T.E. 1988. Low levels of genetic variability of yellow perch (*Perca flavescens*) in Lake Erie and selected impoundments. *In* The biogeography of the island region of western Lake Erie. *Edited by* J.F. Downhower. Ohio State University Press, Columbus, Ohio. pp. 246–257. Available from https://kb.osu.edu/dspace/handle/1811/24663 [accessed 20 July 2011].

Swatdipong, A., Vasemägi, A., Koskinen, M.T., Piironen, J., and Primmer, C.R. 2009. Unanticipated population structure of European grayling in its northern distribution: implications for conservation prioritization. Front. Zool. **6**: 6. doi:10.1186/1742-9994-6-6.

Todd, T.N., and Hatcher, C.O. 1993. Genetic variability and glacial origins of yellow perch (*Perca flavescens*) in North America. Can. J. Fish. Aquat. Sci. **50**(9): 1828–1834. doi:10.1139/f93-205.

Tyson, J.T., and Knight, R.L. 2001. Response of yellow perch to changes in the benthic invertebrate community of western Lake Erie. Trans. Am. Fish. Soc. **130**(5): 766–782. doi:10.1577/1548-8659(2001)130<0766:ROYPTC>2.0.CO;2.

Tyson, J.T., Stein, R.A., and Dettmers, J.M. (*Editors*). 2009. The state of Lake Erie in 2004. Gt. Lakes Fish. Comm. Spec. Publ. 09-02. Great Lakes Fishery Commission, Windsor, Ont. Available from http://www.glfc.org/pubs/SpecialPubs/Sp09_2.pdf [accessed 20 July 2011].

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes, **4**(3): 535–538. doi:10.1111/j.1471-8286.2004.00684.x.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2006. MICRO-CHECKER. Available from http://www.microchecker.hull.ac.uk [accessed 20 July 2011].

VanDeHey, J.A., Sloss, B.L., Peeters, P.J., and Sutton, T.M. 2009. Genetic structure of lake whitefish (*Coregonus clupeaformis*) in Lake Michigan. Can. J. Fish. Aquat. Sci. **66**(3): 382–393. doi:10.1139/F08-213.

Waples, R., Punt, A., and Cope, J. 2008. Integrating genetic data into management of marine resources: How can we do it better? Fish Fish. **9**(4): 423–449.

Wei, A., Chow-Fraser, P., and Albert, D. 2004. Influence of shoreline features on fish distribution in the Laurentian Great Lakes. Can. J. Fish. Aquat. Sci. **61**(7): 1113–1123. doi:10.1139/f04-061.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**(6): 1358–1370. doi:10.2307/2408641.

Wilson, A., Arcese, P., Keller, L.F., Pruett, C.L., Winker, K., Patten, M.A., and Chan, Y. 2009. The contribution of island populations to in situ genetic conservation. Conserv. Genet. **10**(2): 419–430. doi:10.1007/s10592-008-9612-3.

YPTG. 2006. Report of the Lake Erie Yellow Perch Task Group. Yellow Perch Task Group, Lake Erie Committee, Great Lakes Fishery Commission, Windsor, Ont. Available from http://glfc.org/

lakecom/lec/YPTG_docs/annual_reports/YPTG_report_2006.pdf [accessed 20 July 2011].

YPTG. 2007. Catch limits set for walleye and yellow perch for 2007. Yellow Perch Task Group, Lake Erie Committee, Great Lakes Fishery Commission, Windsor, Ont. Available from http://glfc.org/pressrel/pr070323.pdf [accessed 20 July 2011].

YPTG. 2008. Status of yellow perch in Lake Michigan and Yellow Perch Task Group progress report. Report to the Lake Michigan Committee. Yellow Perch Task Group, Lake Michigan Committee, Great Lakes Fishery Commission, Niagara Falls, Ont. Available from http://in.gov/dnr/fishwild/files/fw-YPTG_annual_2008_final.pdf [accessed 20 July 2011].

YPTG. 2009. Annual report of the Lake Erie Yellow Perch Task Group. Yellow Perch Task Group, Lake Erie Committee, Great Lakes Fishery Commission, Windsor, Ont. Available from http://glfc.org/lakecom/lec/YPTG_docs/annual_reports/YPTG_report_2009.pdf [accessed 20 July 2011].

YPTG. 2010. Annual report of the Lake Erie Yellow Perch Task Group. Yellow Perch Task Group, Lake Erie Committee, Great Lakes Fishery Commission, Ann Arbor, Mich. Available from http://glfc.org/lakecom/lec/YPTG_docs/annual_reports/YPTG_report_2010.pdf [accessed 20 July 2011].

YPTG. 2011. Annual report of the Lake Erie Yellow Perch Task Group. Yellow Perch Task Group, Lake Erie Committee, Great Lakes Fishery Commission, Ann Arbor, Mich. Available from http://www.glfc.org/lakecom/lec/YPTG_docs/annual_reports/YPTG_report_2011.pdf [accessed 20 July 2011].

Contribution of local fishermen to improving knowledge of the marine ecosystem and resources in the Republic of Guinea, West Africa

Jean Le Fur, Athanase Guilavogui, and Antoine Teitelbaum

Abstract: We assessed the quality of fishermen's local ecological knowledge, or LEK, as a potential source of information about coastal ecosystem functioning in the Republic of Guinea. Interviews were conducted by means of partial immersion or repeated surveys at six landing sites. In each site and for each topic, discussions were conducted with 3 to 15 individual fishermen and 1 to 10 groups of fishermen. Knowledge was obtained about habitats, substrate preferences, the location of nurseries, reproductive cycles, fish diet, and the trophic network of the Sciaenid community, the major resource for fisheries in this area. We systematically compared the reliability of the information collected with that of scientific information collected in parallel surveys or published data. The contribution of LEK should be considered on a case-by-case basis. Indeed, LEK could be used as (*i*) a supplementary source of scientific studies (seabed description), (*ii*) a basis for new scientific investigation (species reproductive cycle), (*iii*) the only possibility to obtain information (nursery location), (*iv*) a surrogate to scientific surveys providing an identical level of validity (fish diets) or a satisfactory proxy (trophic network) in a context of limited resources and data in which wide-ranging knowledge relating to the entire coast must be obtained.

Résumé : Nous caractérisons l'étendue et la qualité du savoir écologique des pêcheurs (SEP) comme source potentielle de connaissances sur l'écosystème de la zone côtière de la république de Guinée. Le SEP a été obtenu par immersion partielle ou enquêtes répétées dans six centres de débarquement. Dans chaque site et pour chaque sujet, les enquêtes ont été réalisées sur un ensemble de 3 à 15 pêcheurs individuellement et de 1 à 10 groupes de pêcheurs interrogés simultanément. Les SEP obtenus concernent les habitats, les préférences édaphiques, la localisation des nourriceries, les cycles reproductifs, le régime alimentaire et le réseau trophique de la communauté à sciaenidés, la principale ressource démersale dans cette région. Les SEP obtenus ont été systématiquement associées, lorsque c'était possible, à une information scientifique équivalente collectée en parallèle ou issue de la littérature. Le SEP peut être utilisé selon les cas comme (*i*) source complémentaire aux études scientifiques (description des habitats), (*ii*) inspiration pour de nouvelles recherches (cycles reproductifs des espèces), (*iii*) seule source possible de connaissance (localisation des nourriceries), (*iv*) substitut aux études scientifiques dans un contexte de validité similaire (régimes alimentaires) ou comme un proxy acceptable (réseaux trophiques) dans un contexte limité en moyens et en données disponibles pour lequel une variété de connaissances doivent être acquises sur l'ensemble de la zone côtière.

Introduction

Fisheries management and research nowadays focus strongly on an ecosystem approach to fisheries (EAF; Browman and Stergiou 2004; Garcia and Cochrane 2005; Pitcher et al. 2009), promoted alongside similar approaches (Garcia et al. 2003), such as ecologically sustainable development (Fletcher 2006) and ecosystem-based fishery management (Christie et al. 2007; Marasco et al. 2007). In addition to fishery, governance, or social-related issues, these new approaches require a "resource ecology" perspective with consideration of the physical, nutritional, and competitive natural environment in which marine resources develop (Garcia and Cochrane 2005; Rice 2008; Zhang et al. 2009).

An EAF is now considered necessary for all fishery systems, including those of developing countries (Sherman and Duda 1999; Sinclair et al. 2002), for which the adoption of this approach is a major issue (Mathew 2003; Heck et al. 2007). This is the case in the Republic of Guinea, whose coastal area has highly productive demersal fishery resources and is subject to major threats (Domain et al. 2000; Sidibe 2003; Ukwe et al. 2006). Guinea is currently in the process of defining the conservation issues it faces with the establishment of wetland sites of international importance under the

Received 14 June 2010. Accepted 30 March 2011. Published at www.nrcresearchpress.com/cjfas on 19 August 2011.
J21875

Paper handled by Associate Editor Marie-Joëlle Rochet.

J. Le Fur. Institut de Recherche pour le Développement, Centre de Biologie et de Gestion des Populations (INRA/IRD/Cirad/Montpellier SupAgro), Campus international de Baillarguet, CS 30016, F-34988 Montferrier-sur-Lez cedex, France.
A. Guilavogui.[†] Centre National des Sciences Halieutiques de Boussoura (CNSHB), BP 3738/39, Conakry, Guinea Republic.
A. Teitelbaum. SPC (Secretariat of the Pacific Community), BP D5, 98848 Noumea CEDEX, New Caledonia.

Corresponding author: Jean Le Fur (e-mail: lefur@ird.fr).

[†] Deceased.

Can. J. Fish. Aquat. Sci. **68**: 1454–1469 (2011) doi:10.1139/F2011-061 Published by NRC Research Press

Ramsar Convention (Ramsar Convention Bureau 1997 in Seto and Fragkias 2007) and the identification of marine protected areas (International Union for Conservation of Nature 2007, 2008).

However, to ensure efficient EAF implementation (Mathew 2003; Rice 2008), this country needs to accumulate large amounts of ecological and social information. For example, an EAF may involve the identification of marine protected areas (Browman and Stergiou 2004; Garcia and Cochrane 2005; Greenstreet et al. 2009) as potential conservation zones (Sissenwine and Murawski 2004). As far as the specific focus of knowledge about the ecosystem is concerned, this would require an inventory of current ecological communities, vulnerable species, critical habitats and their relationships, resource life traits, and trophic relationships for the entire shoreline and possibly the entire ecosystem. In this situation, fishermen's local ecological knowledge (LEK; Olsson and Folke 2001; Davis et al. 2004) may be a valuable source of information, addressing the problem of the diverse nature and quality of the ecological knowledge required.

Since the pioneering work of Johannes (1981, 1984), the intrinsic value of local operators' knowledge of the ecosystem within which they work has been increasingly recognized (Davis and Wagner 2003; García-Allut et al. 2007) and used. Study protocols have been refined, with comparative approaches between countries (Folke 2004; Silvano and Begossi 2005) or precise quantification of the knowledge acquired (Moreno et al. 2007, Silvano and Begossi 2010). The traditional knowledge of fishermen is now used for many purposes, including the deciphering of ecological interactions (Folke 2004) and studies of trends (Neis et al. 1999) and changes (Johannes et al. 2000; Gullström et al. 2006). This knowledge is also used to deal with questions concerning particular taxonomic groups (Davis et al. 2004; Silvano and Begossi 2005; Grant and Berkes 2007) or specific issues, such as fish aggregation (Moreno et al. 2007), habitats (Bergmann et al. 2007), trophic relationships (Pikitch et al. 2004), or the identification of sampling areas (Davis et al. 2004). Finally, various cultures and traditions concerning fishing activity have been shown to be based on a structured corpus of knowledge (Hickey 2007; Houde 2007). In addition to simply providing a reservoir of facts (Baelde 2007), this knowledge may be determinant when trying to resolve questions relating to conservation measures or sound management design (Hickey 2007; Berkes 2008).

The use of LEK has been described as an asset for the implementation of an EAF (Garcia and Cochrane 2005; Gray and Hatchard 2008; Paterson and Petersen 2010). However, the practical use of LEK, although increasing (Prigent et al. 2008; Silvano and Valbo-Jorgensen 2008; Gerhardinger et al. 2009), is not common within this context, and its reliable use is sometimes called into question (e.g., Davis and Wagner 2003; Haggan and Neis 2007). Indeed, many studies have focused on the relationships and associations between local knowledge and "modern scientific knowledge" (Johannes and Neis 2007). Most recognize the complementary nature of LEK (García-Allut et al. 2007; Williams and Bax 2007) to various degrees: separate from (Wilson et al. 2006; Symes 2008), complementary to (Silvano et al. 2005; Baelde 2007; Hall et al. 2009), or enhancing (D'Incao and Reis 2002; Aswani and Lauer 2006; Berkes et al. 2007) scientific knowledge. The reliable use of local ecological knowledge as a basis of management decisions also remains a matter of debate, and various approaches for increasing its validity have been proposed (Davis and Wagner 2003; Maurstad et al. 2007).

We evaluated the potential and reliability of this source of knowledge as a means of improving ecological knowledge acquisition in the development of an EAF. This study was carried out along the coast of Guinea, which boasts a large community of fishermen (Chavance 2000) with potentially valuable knowledge for the identification of sensitive areas, such as areas of marine resource renewal. Wide-ranging ecological knowledge was obtained from fishermen and compared with knowledge from equivalent scientific sources. We focused on general perception of the environment (seasons, habitats, and biotopes), particular knowledge about reproduction (location of nurseries, reproductive cycle), and feeding (diets of fish, trophic network). We present the approach developed and the knowledge provided together with the equivalent scientific information obtained either from surveys carried out in the field during the study or from published data. We then consider the quality of LEK and scientific results for improving knowledge about the coastal ecosystem.

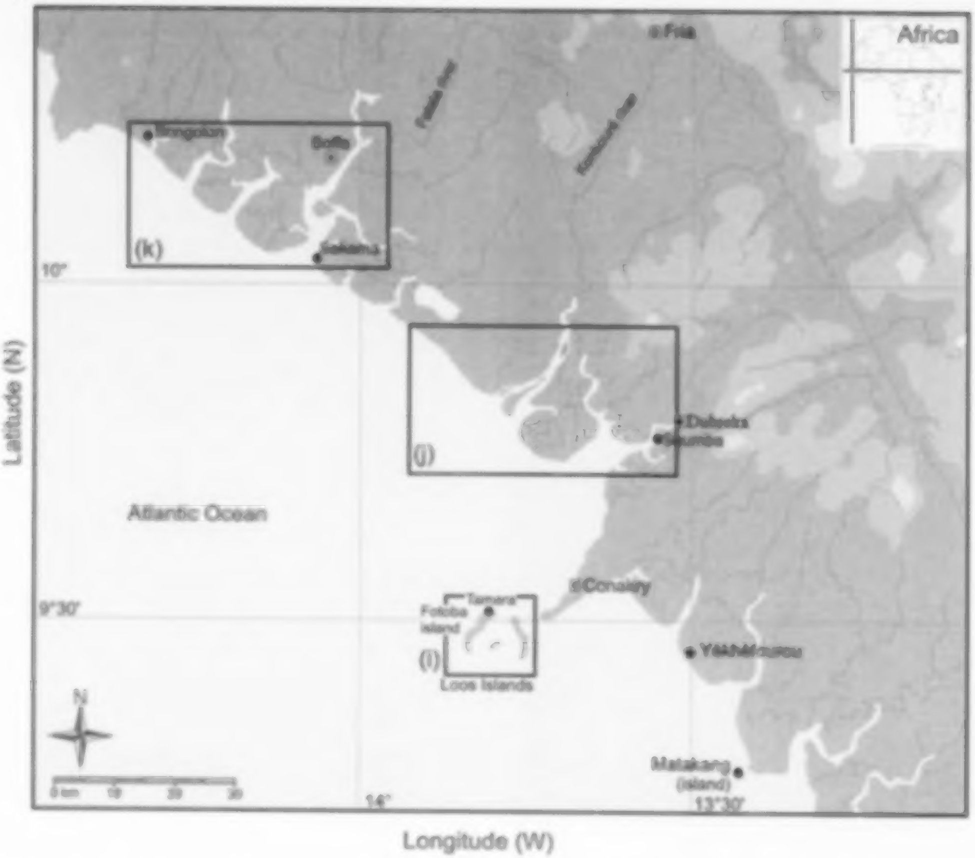
Materials and methods

Study site and the population of fishermen surveyed

The coastal ecosystem of Guinea is a large delta complex composed of a collection of plains divided by the estuaries of a large number of water courses. It is a highly productive zone enriched in coastal silt from the rivers. According to Saenger and Hegerl (1983), conditions in this area have favoured the development of one of the largest mangrove forests in Africa, which, within a complex trade-off between positive and negative factors (Baran 2000), play a major role as nursery areas for most of the fish species harvested. Within this ecosystem, a major demersal assemblage of fish species, known as the "Sciaenid community" (Longhurst 1966), named after the predominant family present, develops (Domain 1989 in Domain et al. 2000). This very diverse community (18 families, 38 species) is closely associated with the muddy bottoms and desalinated waters of estuaries (Sidibe 2003). It moves back and forth between the estuaries and the open sea, with complex dynamics (Baran 1995). The Sciaenid community is the main source of fish for industrial (Lesnoff et al. 2000) and artisanal (Chavance 2000) demersal fisheries in Guinea. However, many gaps remain in our bio-ecological knowledge of this community and its potential sustainability, given the high level of fishing to which it is subjected (Baran 2000). The main features requiring clarification concern trophic relationships between the fish communities and the estuarine environment and the conditions in which reproduction occurs and juvenile stages develop (Baran 1995).

The fishing industry in Guinea can be subdivided into an industrial fleet of 200 boats, mostly run by foreign companies, and a small-scale fishery of 2500 wooden canoes of various types, 41% of which are motorized (Sidibe 2003). These boats are distributed between 120 identified landing sites (Chavance 2000) of various sizes, mostly embedded within the numerous mangrove estuaries along the coast. A

Fig. 1. Location of the study sites for the two survey phases. Black dots indicate the landing sites at which the surveys were conducted. Inset (*i*) corresponds to Fig. 3; insets (*j*) and (*k*) correspond to Fig. 4.



chronic situation of conflict has been described in which illegal industrial vessels repeatedly penetrate into the areas reserved for small-scale fisheries (Guilavogui et al. 2004). The coastal demersal stocks were almost entirely unexploited before 1985. Following the establishment of the republic and the liberalization of fishing, there has been a large and rapid increase in fishing pressure over the last 25 years (Gascuel et al. 2008). Fish stocks are now threatened, with demersal marine resource abundance and the mean length of species now only a third their values in 1985 (Sidibe et al. 2004).

The study took place in the central coastal areas of Guinea (Fig. 1) and was divided into two phases. The first phase was carried out in 1999 in the Loos Islands, a small archipelago off the Conakry Peninsula. These fish-rich islands are a traditional fishing zone. The study focused on the harbour of Tamara on the island of Fotoba. This island had a population of 39 fishermen, comprising native fishermen of the Soso ethnic group and migrant fishermen who arrived from Sierra Leone in the 19th Century. These fishermen use drift nets, trammels, floating nets, or fishing lines.

The second phase of the study took place in 2001–2003 in the principal estuaries in the central coastal area of Guinea. These estuaries are bordered by mangroves and are representative of the coast in this region. Five harbours used for the unloading of catches by local fishermen were studied by means of repeated surveys in this phase of the study: Bongolon, Sakama, Soumba, Matakang, and Yèkhèfourou. These harbours were selected for study on the basis of the large number of fishing boats unloading there and the long tradition of fishing at these sites. Fishing boat captains and former fishermen were selected for the survey; less than 10% of those interviewed were literate.

Survey design and interview strategy

Integration into the fishermen's environment

Johannes (1981) established the fundamental principles for obtaining high-quality local knowledge. The principal requirement is a "socio-ecological" approach, established such that fishermen are placed at their ease. These principles (adapting working hours to those of the fisherman interviewed, avoiding brief interviews or visits) led to a survey protocol based on two different communication approaches.

The first phase of the study (Loos Archipelago) was carried out by a French biologist (A. Teitelbaum) who stayed in the fishing village for 6 weeks and in the lives of the fisherman on land and at sea. Within this phase, the most suitable form to interview fishermen was a mixture of "meetings

Table 1. Number of interviews with fishermen conducted for each of the subjects studied.

	Survey sites						
	Phase I	Phase II					
	Loos	Matakang	Yèkhèfourou	Soumba	Sakama	Bongolon	Total phase II
Description of seasons	—; —	3; 1	4; 2	—; 5	—; 2	—; 3	7; 13
Nature of the seabed	15; 10	—; —	—; —	7; 5	10; 2	6; 3	23; 10
Prefered biotopes	15; 10	3; 1	4; 2	7; 5	10; 2	6; 3	30; 13
Nursery areas location	—; —	—;—	—; —	7; 5	10; 2	6; 3	23; 10
Reproduction cycle	—; —	—; 1	—; 2	7; 5	10; 2	6; 3	23; 13
Fish diets	15; 10	3; 1	4; 2	7; 5	10; 2	6; 3	30; 13
Trophic networks	—; —	3; 1	4; 2	7; 5	10; 2	6; 3	30; 13

Note: Within each cell, the first value corresponds to the number of fishermen interviewed individually; the second value is the number of groups of fishermen interviewed during the survey at the given site. The number of active fishermen within each group varied (some arriving, some leaving, some talking, some being silent) and was not reported. Each group contained from 4 to about 20 fishermen.

under a tree" lasting about 1 hour with either one or several fishermen, full-day meetings sharing the life of a fisherman or fishing crew on the boat (10 different fishing crews), and several weeks of interaction with the fishermen with whom the scientist was staying (trips out to sea, accommodation, meals). We also approached fishermen not permanently based on the island (mostly dive fishing), most of whom came from the Conakry Peninsula.

Phase II was carried out in estuaries by a Guinean researcher (A. Guilavogui); for each visited site, the same approach was repeated: following their arrival (by car or by canoe), the scientific team met with the village chief and informed him of the aim of their stay. The chief then designated himself or a guide to introduce the team to designated knowledgeable fishermen (old fishermen and captains). During their stay, the team members could also ask to meet other people (snowball sampling). Apart from this procedure, the terms and conditions of meetings (number of meetings and their length) with fishermen or groups of fishermen depended on fishing activities and the availability of the fishermen. Overall, this phase consisted of a series of six stays of 5–6 days at a time, each involving a partial immersion in village life. During phase II and for each of the areas of knowledge considered, we questioned 7 to 30 fishermen (Table 1) between the ages of 30 and 70 years in individual surveys, in which fishermen freely expressed their knowledge on the subjects considered. A second approach was used in which several fishermen were brought together to discuss specific subjects. The results presented for each subject are a final compilation of the various notes taken during the surveys.

Semidirected interviews

Detailed questionnaires prepared in advance proved unworkable because the series of questions clearly disturbed some of the fishermen interviewed. Many appeared determined, at all costs, to provide an answer to any question posed, and this approach may therefore entail a risk of discrepancy between the fisherman's true knowledge and the answers given. We optimized the quality of the accounts given by fishermen by taking notes, using questionnaires only to guide the interviews, and to identify the subjects to be considered.

Use of intermediaries

In both phases of the study, one or two individuals trusted by the fishermen and capable of translating between fishermen and researchers were employed as intermediaries. In phase I, an interpreter (A. Sökhö Camara) living in the village was used. A. Teitelbaum already had basic competence in the local language. In phase II of the study, an interviewer–interpreter (R. Manet Karemba) from the research centre, who had previously worked in a fishing crew in the area, served as an intermediary. The choice of intermediary seemed to be crucial, affecting the way in which questions were posed, the reconstitution of responses, and the likelihood of meeting people with relevant knowledge (Johannes et al. 2000; Davis and Wagner 2003). This contribution was qualitative and could not be quantified. The intermediary may, for example, account for cultural misinterpretation and provide real added value in terms of the worth of the data collected (Ames 2007).

Use of maps

Map drawing has been described as a good way to interact with fishermen (Bergmann et al. 2007; Wilson et al. 2006). During phase I, the fishermen could read scale maps of their fishing territories with no particular difficulty; during phase II, after some training, fishermen were able to interpret the views of their fishing zones from above (Aswani and Lauer 2006). They could thus locate the density of fish or the nature of the seabed.

Subject areas included in the study

A set of domains of information (environment, resources, and their relationships) linked to the Sciaenid community was investigated during the surveys. Information domains were selected on the basis of their value for both understanding the Guinean marine ecosystem functioning and evaluating rigor and confidence in the results obtained, since the purpose of the study was to confront LEK and equivalent scientific knowledge. We present within this section the subject areas included in this work, together with the way in which the corresponding scientific information was obtained, where possible.

Perception of time

For each season, we characterized the criteria defining the climatic situation, fishing practices, and the abundance and succession of marine organisms. This knowledge was extracted from group discussions in the second phase of the study and compared with the usual types of seasonal segmentation of the year.

Nature of the seabed

Most of this knowledge is gained from observation of the samples of seabed brought to the surface by their fishing gear. This became especially clear during the first phase of the study on the Loos Archipelago, where the seabed is more heterogeneous than that in the mangrove. The research team accompanied the fishermen in their boats 4 days per week, on average, during the study period. During these outings, the nature of the seabed was determined and cross-checked by bucket or anchor sampling (for silt) or diving when conditions were favorable. Each fishing crew helped to complete, on board, an outline map of the islands (same scale and size as Fig. 3) based on its own usual fishing zone (dependent on the fishing gear used and the target species). The resulting overall map is a synthesis of maps developed over the 6-week stay of phase I. Zones were identified with maps and by GPS. These results were compared with data extracted from the general map proposed by Domain and Bah (1993).

Biotopes

Preferences of the organisms for particular substrates were systematically addressed during the interviews at the various sites studied in phases I and II; fishermen were asked about the type of substrate on which each demersal species they targeted was found. We also cross-checked the information obtained with published results concerning the Guinean area.

Fish reproduction

The nursery zones of the principal species exploited were identified for the two principal estuaries of Fatala and Konkoure. The overall distribution was determined by putting together all the accounts recorded in the participatory processes (Table 1), with base maps used as a medium of communication for each survey site concerned (Bongolon and Sakama for the Fatala estuaries and Soumba for the Konkoure estuary). After discussion on land, canoe trips were organized to position, with a GPS device, the areas described by fishermen.

The reproduction cycle also was investigated in phase II on the basis of collective and individual interviews. Results for the bonga shad (*Ethmalosa fimbriata*) were compared with the reproductive cycle described by Baran (1995) for the Fatala estuary.

Fish diet

Fishermen routinely open the fish they catch, principally during the operations carried out during the treatment of the catch. This provides them with information about the stomach content and diet of the fish. Fishermen were systematically asked about the feeding habits of the fish they targeted. For this topic, questions were of the kind, "What do you find when you open the belly of the fish?". We present results only for affirmations that we were able to confirm at more than one survey site.

In parallel, we compared fishermen's knowledge of the diet of the fish they caught with the results of stomach content analyses carried out on site during the first phase of the study. The scientific analysis was carried out as follows: after each fishing trip, fish gut contents were analyzed immediately on landing, after their purchase, or with the agreement of the fish buyers. The fish were identified and classified to species level, measured, and weighed. The contents of their stomachs were analyzed with a binocular lens and an electronic balance. The repletion status of the fish was scored, together with the amounts of each type of food and the frequencies of occurrence. We retained the results obtained only for taxonomic groups for which at least 50 stomachs were analyzed. The results were then used for an overall synthesis of all the stomachs studied and converted into "presence–absence" format for comparison with the statements of the fishermen. Stomach contents were analyzed in October, at the end of the rainy season. The food regimes are therefore not entirely representative of the nutrition of these species generally throughout the year.

Reconstruction of the trophic network

For this subject, the initial questions were of the kind, "What do the fish eat?", posed species by species, starting with major species of the Sciaenid community. More often than not, the discussion moved on to an informal description of the global network by fishermen or groups. Each question was debated by fishermen, and the discussion then led to a description of the global families (trophic levels) of fishes within the ecosystem. The trophic network question was addressed at all study sites during phase II surveys and systematically discussed during the group interviews. The notes taken on this topic were collated at the end of the surveys. The results are presented as an integrated overview, bringing together the various accounts. The resulting trophic network therefore reflects the major trophic relationships and does not contain rarely recorded taxonomic groups or details that varied with the fishermen interviewed and the location.

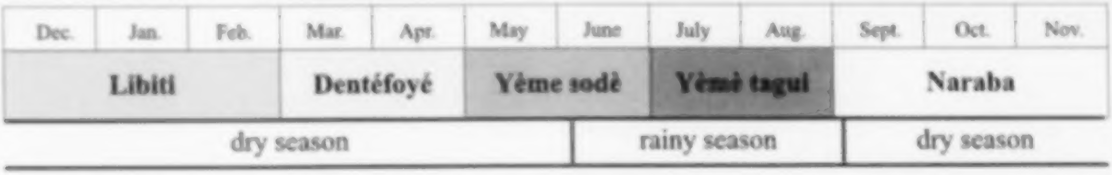
The descriptions of the trophic network were compared with the results of a trophic network model developed for the Guinean marine ecosystem by Guénette and Diallo (2004*a*, 2004*b*) using the Ecopath approach (Christensen and Pauly 1992). This method uses estimates of the proportion of each prey in the consumers' diet based on a trophic mass balance analysis of the global ecosystem (Christensen and Walters 2004). In their model, Guénette and Diallo (2004*a*) have reconstructed diets from composite bibliographic sources. The Ecopath model also deals with a large number of taxa, but we considered only those species discussed by the fishermen.

Results

Ecological segmentation of the year

The ecological segmentation of the year by fishermen differed from standard classifications. The local fishermen knew the 12 months of the Gregorian calendar. They grouped these months together into distinct seasonal periods (Fig. 2). Each period was defined by criteria dependent on variations in the physical parameters of the marine and coastal environment. Their classification was based principally on wind and rainfall conditions. The interviews also indicated that these seasons took into account the characteristics of currents, atmospheric visibility, and sunshine intensity, as well as the

Fig. 2. Comparison of the scales of temporal observation of Guinean fishermen (middle) with the Gregorian calendar (top) and the progression of seasons in the tropical zone (bottom).



temperature, salinity, and turbidity of the water. The fishermen related their segmentation of the year to the behaviour of the fish, including the arrival and departure of fishing resources. Five principal periods were identified: two of 3 months and three of 2 months (Fig. 2).

"Libiti" (December, January, February) is a calm period characterized by inshore breezes. Sunshine intensity is weaker during this period, and many species migrate towards the seabed. Drift-net fishing is therefore effective. Large, seabed-dwelling shrimps (*Parapenaeopsis atlantica*, *Penaeus notialis*) are abundant, and groups of common dolphins (*Delphinus delphis*) are observed in the coastal areas, where they hunt fish.

"Denté foyé" (March, April) is characterized by the arrival, generally in the afternoon, of strong offshore winds. These winds are described by the fishermen as corresponding to the peak of the continental trade winds blowing from the east to the northeast (Pezennec 2000). The water is warm both at the surface and at the seabed, and most fish migrate further out to sea, obliging the fishermen to travel large distances to reach them. This is the start of the sardinella (*Sardinella maderensis*) season and also corresponds to the first appearance of jellyfish (Leptomedusae and Limnomedusae suborders; "yébognè" in Soso). Fishing trips are limited during this period and accidents are frequent.

"Yèmè sodè" (May, June) corresponds to the start of the rainy season. This period is characterized by tornados, known as "tornadè foyé", and signals the end of the "denté foyé" or coastal winds. The wind triggers the migration of pelagic fish to the coastal zone, favouring the formation of large shoals of fish. This is the major fishing period (also named the "miraculous" fishing season by fishermen). Some fishermen think that in addition to the effects of the wind, the thick clouds forming on the coast might attract the fish. This period is also characterized by the migration of marine sea turtles (various species from the Chelonioidea superfamily) to the coastal zone. Some fishermen think that the turtles come to reproduce, whereas others think that they come to feed on jellyfish, which are also abundant during this period.

"Yèmè tagui" (July, August), in the middle of the rainy season, is characterized by a large decrease in the salinity of coastal waters and a large increase in turbidity. Certain species, such as the bonga shad (*E. fimbriata*) move further out to sea.

"Yèmè donkhoè" or "naraba" (September, October, November) corresponds to the end of the rainy season. The sky is blue and the sun shines. There are no violent winds and fishing conditions are good, particularly for the bonga shad. The large species of commercial interest (*Pseudotolithus* spp., Ariidae) are also heavily fished during this period.

Nature of the seabed

In the Loos Islands, we found general agreement between the fishermen participating in the study concerning the nature of the substrate and the positions of the various zones. This made it possible to construct a map of the nature of the seabed in this area. Fishermen identify three types of seabed around the Loos Islands (Fig. 3*a*). First, sandy seabeds take the form of (*i*) pure sand with a phenomenon known as "the river" ("ribakoui" in Soso) and comparable to underwater sand dunes, consisting of a large strip of pure, nonsilty sandbank about 10 m below the water surface, (*ii*) sand banks, and (*iii*) sandy seabeds. The second type is a mixture of sand and silt, known as "poto-poto". This mixture is divided into three classes: pure silt, sandy silt, and silty sand. Moreover, vernacular expressions describing the degree of "stickiness" of the poto-poto provide a continuous qualitative reflection of the sand–silt mixture. Rocky seabeds constitute the third type.

This set of classifications is compared with that proposed by Domain and Bah (1993). These authors also used a classification for sand and one for silt, expressed in lutite content. The cross-referencing of these two classifications led to the definition of five classes for the area studied (Fig. 3*b*), to which the classes recognized by the fishermen on the basis of sand and silt content can be related.

Good concordance was found between the seabed structures identified with the two approaches in the area studied. The main differences were the lower proportion of sandy silt identified by fishermen and the greater detail provided by fishermen overall. For example, rocks are also locally identified as grey or red rocks and generally divided into visible rocks and sunken rocks, which are themselves broken down into isolated rocks, rocky ridges, and highly rocky seabeds not shown on the map. In the estuarine area (second phase of the study), LEK also provided another vernacular classification of silt based on color and consistency: soft silt (bolbola), black silt (bora fore), red silt (bora ghéli), and whitish silt (bouibora or bora fikhè).

Seabed preferences (knowledge about biotopes)

In this field, the oldest fishermen with extensive experience of fishing tended to be the most knowledgeable. Fishermen clearly distinguished between different environmental characteristics. For example, according to the fishermen, *Pseudotolithus senegalensis* (boho in Soso) hunts shrimps close to the coast on "hard" silts, whereas *Tilapia* sp. are encountered on white soft silts (borafikhè), where it hunts crabs. The abundance of the bonga shad depends on water dynamics (tide cycle), etc.

We reconstituted the biotic preferences of the various species from the data collected in the surveys and selected only those groups for which comparison with published results was possible (Table 2).

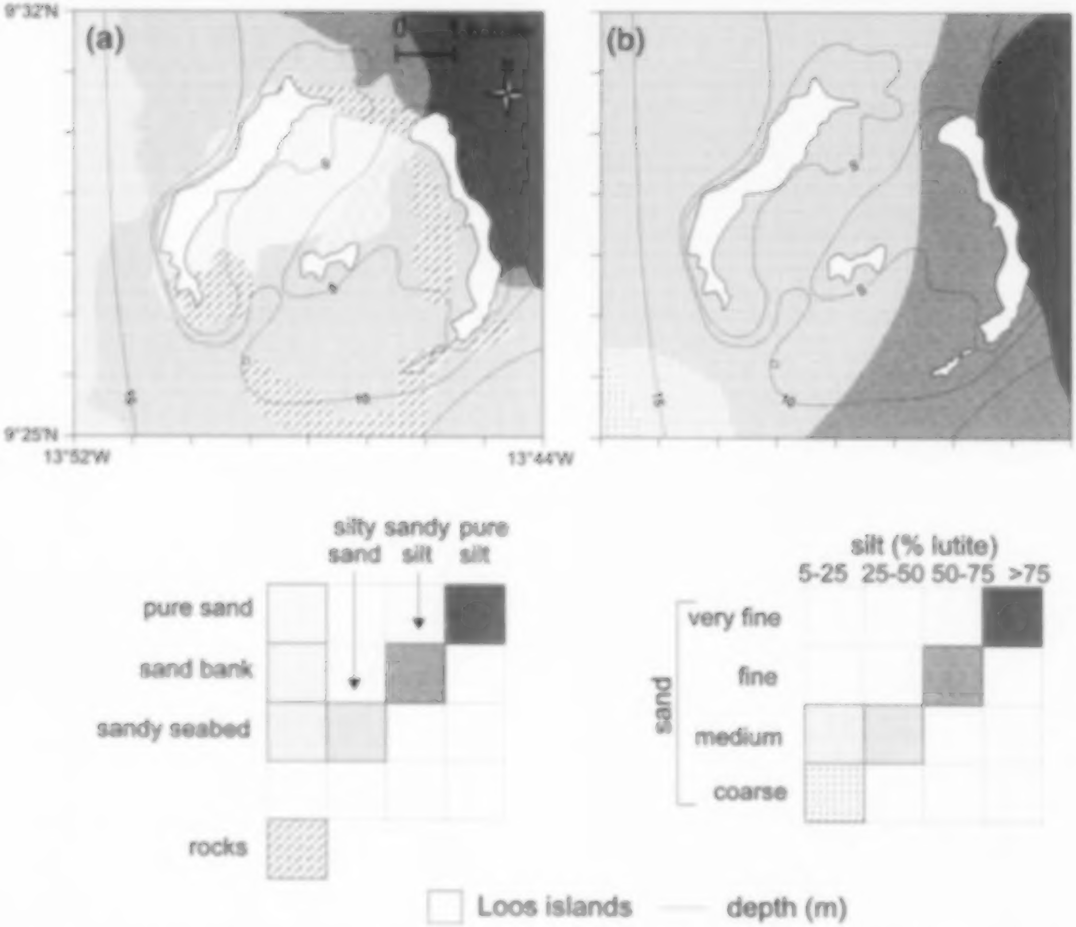


Fig. 3. Seabed map for the Loos Islands and correspondence between local ecological knowledge (LEK) and published knowledge. (*a*) Map constructed from the knowledge of local fishermen and confirmed with control samples. (*b*) Map constructed based on the general seabed map of the Guinean exclusive economic zone by Domain and Bah (1993). The seabed classification grid is the original key from this last source. Map (*a*) is constructed with a grid corresponding as closely as possible to that of map (*b*). 1 nautical mile = 1.852 km.

Knowledge concerning fish reproduction

Location of nurseries

Local fishermen acquire their knowledge of fish reproduction by catching juveniles and observing the physical state of mature fish (e.g., "if the fish isn't pregnant it's flat, with a dry skin. Its belly gets bigger during the laying period", as stated by MS, a retired fisherman from Sakama) and fish morphology during processing operations. Through these observations, the fishermen localize the nurseries and determine the periods of maturation and egg laying. Fishermen precisely located current nurseries for two estuaries within the study (Fig. 4). For the Konkoure estuary (Fig. 4*a*), differences were observed between the two principal estuary arms, with *Pseudotolithus elongatus* widely distributed in the Konkoure arm, whereas the Soumba arm was mostly occupied by *Polydactylus quadrifilis* and *Arius* sp.

Again, in the Fatala estuary area (Fig. 4*b*), the fishermen indicated different distributions of juveniles and reproductive activity for different species between the arms of the estuary. These differences in distribution were particularly marked for *Pseudotolithus elongatus*, *Liza* sp., *Ethmalosa fimbriata* and *Arius* sp., which appeared to colonize different spatial niches. By contrast, a limited area in the northwest of the zone was found to contain a nursery for many species. The fishermen were unable to provide a meaningful interpretation for this situation.

Reproductive cycle

Fishermen identified periods during which mature adults migrated towards the laying zones and periods of juvenile recruitment. Interviews were conducted about the reproductive cycles of 11 species. However, comparison with available scientific data was possible only for the bonga shad within the Fatala estuary. For this species (Fig. 5), the two oldest fishermen interviewed about this topic (KS, aged 70, and MS, of unknown but probably similar age) provided considerable detail about reproduction (e.g., temporary vertical separation of

Table 2. Comparison of knowledge relating to preferences between local fishermen (A, B) and published results (c, d, e, f).

		Nature of the seabed (source)					
Latin name	Local name	Sand	Silt	Hard silt	Rock	Shells	Broad tolerance
Arius sp.	Konkoe	c	e	B	B	B	—
Ilisha africana	Lati	e	B, e	—	B	B	—
Lutjanus sp.	Woli	—	—	—	B, c, e	B	—
Pomadasys jubelini	Kessi kessi	c	A, c	B	B	B	f
Pseudotolithus brachygnathus	Fouta	A, c	c	B	—	B	—
Pseudotolithus elongatus	Bobo	—	B, d, e	—	B	—	—

Note: A, phase I of this study; B, phase II of this study; c, Schneider 1990; d, Diouf 1996; e, Domain et al. 2000; f, Sidibe 2003.

males and females, links between the period of reproduction and the rice harvesting season). These fishermen reported that mature females started to swim up the estuaries at the end of March, the eggs being laid in April and May. After egg-laying, the fry hatch and continue to develop and grow in the mangrove until October. They then migrate towards the estuaries, where fishing takes place from November to March. In March, the individuals not caught by the fishermen swim up the estuaries to complete the cycle. The period of reproduction described by old fishermen differs markedly from that described by Baran (1995) for the same area. This author showed that reproducers of this species were most abundant in September and October, whereas juveniles were most abundant from January to June. Reports of interviews with fishermen in the Fatala study area about observed areas of reproduction confirmed the calendar presented by the older fishermen, but with additional accounts for reproduction in September and October.

Knowledge concerning trophic relationships

Fish diets

Results of the stomach content analysis have been compared with the accounts of the fishermen for the species for which sufficient number of dissections were carried out (Fig. 6). Both sources of information indicated a high proportion of shrimp in the diets of fish from this ecosystem, the essentially piscivorous diet of the highest trophic level target species, the relatively low importance of molluscs in the diet of the fish, the selective diet of *Pseudotolithus elongatus*, and the omnivorous diet of *Drepane africana*.

For 12 of the 70 cells (17%), the analytical results did not agree with the fishermen's declarations: the consumption of molluscs by *Polydactylus quadrifilis* and of benthos by *Pseudotolithus brachygnatus* and *Pseudotolithus typus* or the omnivorous diet of sea catfish (*Arius* sp.). Similarly, fishermen reported the consumption of benthic moss and worms, whereas these food items were not found by analysis of fish stomach contents. Fishermen did not identify a benthophagic diet for *Drepane africana*, whereas such a diet was observed in stomach analyses.

Finally, single accounts were excluded as a result of the protocol used (e.g., the extreme example of a fisherman reporting the presence of a bird in the stomach of a ray, possibly as a result of the ingestion of a dead chick that fell into the mangrove from a tree). Some of the single observations filled in known gaps in our knowledge but were nonetheless not included in this analysis.

Trophic network

In addition to food intake, the fishermen effectively identified the relationships between the feeding habits of the various biological groups and explicitly reconstituted trophic networks. The trophic network constructed from the fishermen's accounts could be compared (Fig. 7) with the equivalent scientific results obtained by Guénette and Diallo (2004*a*, 2004*b*).

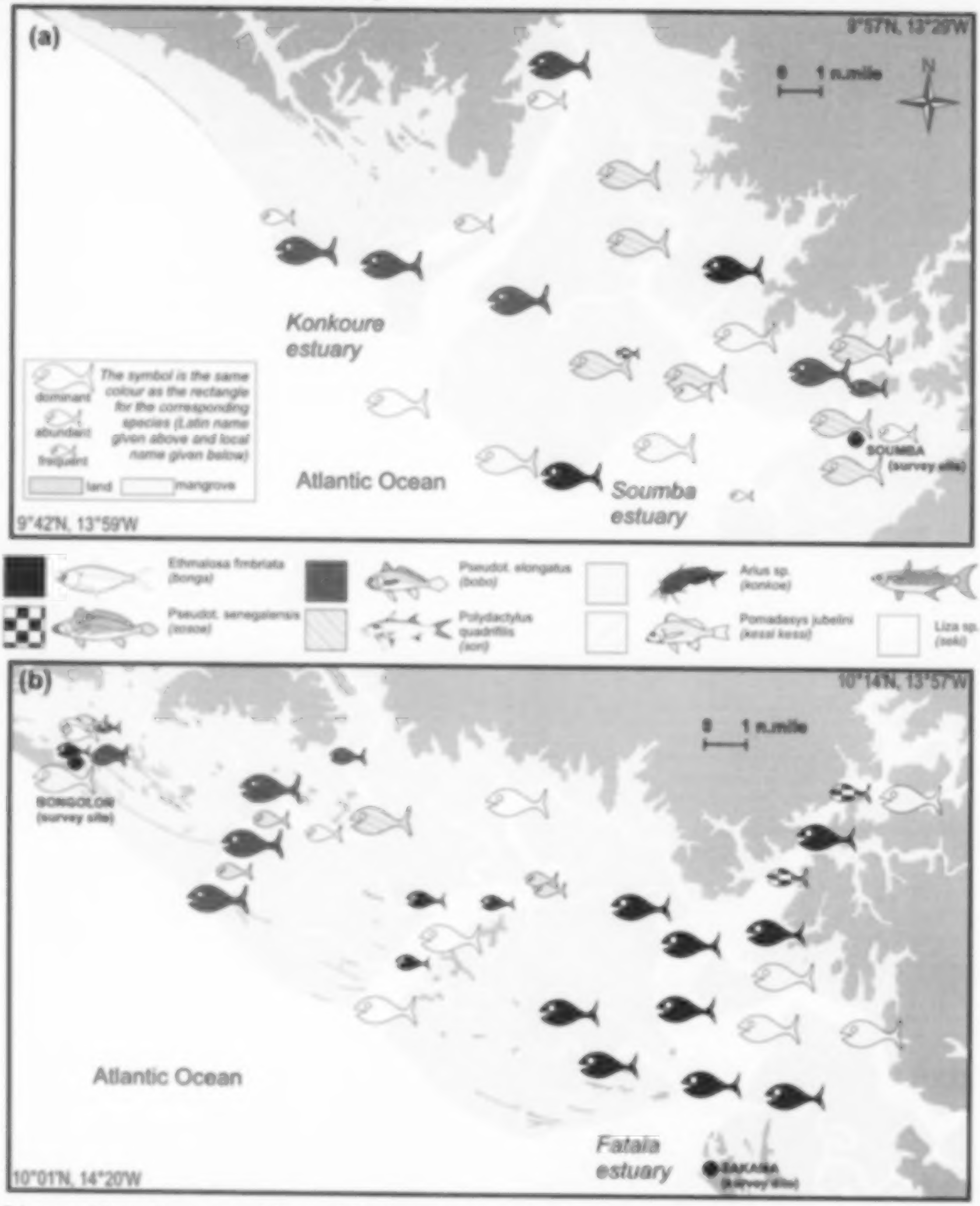
The fishermen identified five categories–levels, differing from standard trophic webs. For comparison purposes, we used the Ecopath scale as a reference similar to that of Lindeman (1942 in Gascuel 2005), with a trophic levels of 1 for primary producers and debris, 2 for secondary producers, 3 for their predators, and a maximum trophic level of 4 to cover top predators.

The fishermen did not consider debris and primary producers to be part of the trophic network. Hence, these elements were coded as level 0 (Fig. 7). In the fishermen's opinion, level I corresponds to organisms (crabs and shrimp) feeding exclusively on the primary substrate consisting of plant debris and silt. This level comprises principally detritus feeders and seems to be similar to reference trophic level 2. Fishermen's trophic level II comprises species feeding on both the organic substrate and individuals at the first level in the food chain. It therefore includes a mixture of detritus eaters and carnivores. This level seems to be intermediate between levels 2 and 3 of the Ecopath classification. Fishermen's trophic level III corresponds to rays and giant African threadfin (*Polydactylus quadrifilis*), which feed on the lower trophic levels. Level IV corresponds to sea catfish (Ariidae), a group of full omnivores. These fish are said to feed on both the elements of the primary substrate and individuals from levels I and II. Finally, level V corresponds to strictly piscivorous fish, such as sharks. These last animals do not feed, in principle, on organisms below level II.

Discussion

The use of two protocols to explore a wide range of topics resulted in the convergence of LEK toward limited but exploitable information about the Sciaenid community, of potential use for the development of an EAF. Conclusive results were obtained about the successive seasonal phases, habitat location, the seabed preferences of groups of species, and the location of nursery areas in the mangroves and estuaries. Fishermen also displayed usable knowledge about the diets of the various species and trophic relationships within

Fig. 4. Location of nursery areas in (*a*) the Konkoure estuary by the fishermen of Soumba (inset "*j*" of Fig. 1), (*b*) the Fatala estuary by fishermen from Bongolon and Sakama (inset "*k*" of Fig. 1). 1 nautical mile = 1.852 km.


(a)
9°57'N, 13°29'W
0 1 n.mile
N
Konkoure estuary
The symbol is the same colour as the rectangle for the corresponding species (Latin name given above and local name given below)
dominant
abundant
frequent
land
mangrove
Atlantic Ocean
Soumba estuary
SOUMBA (survey site)
9°42'N, 13°59'W
Ethmalosa fimbriata (bonga)
Pseudot. elongatus (bobo)
Arius sp. (konkoe)
Pseudot. senegalensis (sosoe)
Polydactylus quadrifilis (sori)
Pomadasys jubelini (kesal kessi)
Liza sp. (seki)
(b)
10°14'N, 13°57'W
0 1 n.mile
BONGOLON (survey site)
Atlantic Ocean
Fatala estuary
SAKAMA (survey site)
10°01'N, 14°20'W

the fish assemblage. Some potentially useful information, such as the timing of reproductive periods, could not be used without complementary research, because the information provided by the fishermen conflicted with the findings of scientific studies.

We focus here on three topics to assess the validity of the information obtained: the biases associated with the approach used, the factors increasing confidence in various types of knowledge, and a comparison of the reliability of LEK and scientific knowledge.

Fig. 5. Differences between scientific and local knowledge concerning the reproduction cycle of the bonga shad in the Fatala estuary. (*a*) Baran (1995) bimestrially estimated frequencies (adapted from the original). (*b*) Qualitative synthesis of the narratives of the oldest fishermen from Sakama. When fishermen described a migration or trend, their accounts were transposed into a progressive decrease or increase; bars indicate observed reproduction spots described by fishermen at the Bongolon and Sakama survey sites. In both panels, dark gray shading is for reproducers, and light gray shading is for juveniles.

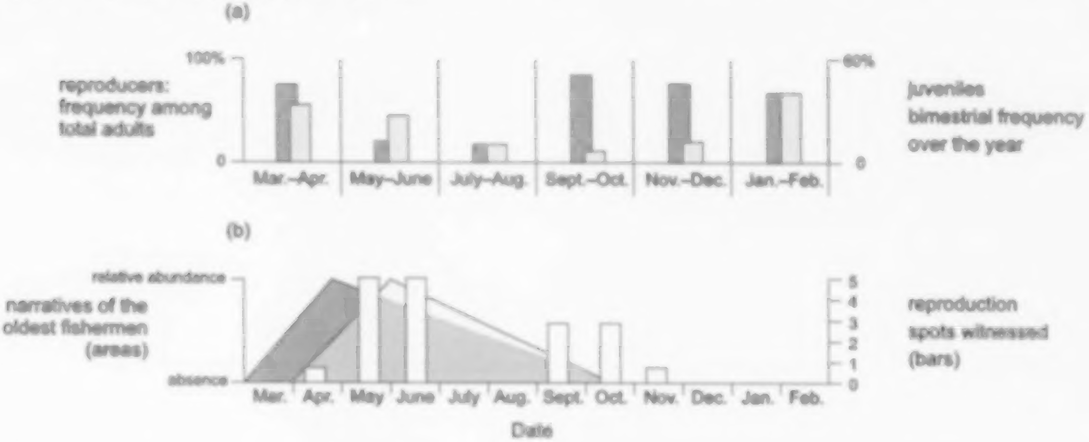
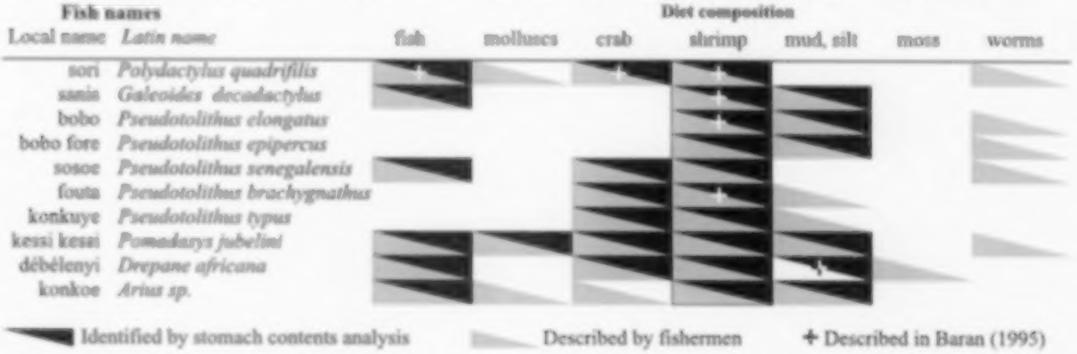
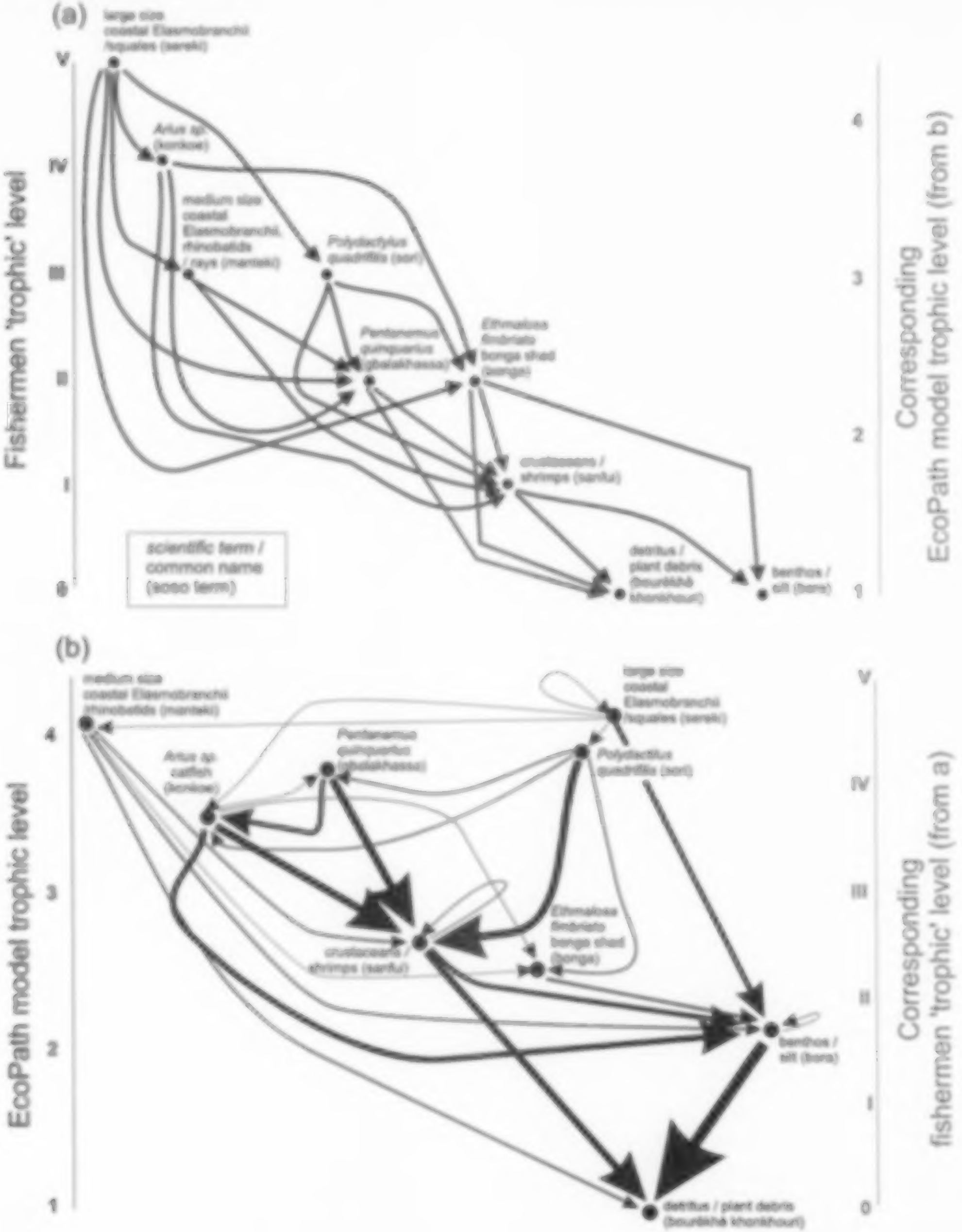


Fig. 6. Comparison of the diets of fish described by the fishermen and those deduced from the analysis of stomach contents.



A key characteristic of the LEK approach is that knowledge was not obtained by direct data sampling but by using fishermen's reports, which, even if recorded in a standard way, may still be biased. One potential source of discrepancy was the differences in language used by the respondents. For example, "grand capitaine" is the name given to *Polydactylus quadrifilis* by fishermen of the Soso ethnic group, whereas this same name is used by fishermen from Sierra Leone within the same port to designate *Pseudotolithus brachygnatus*. Subtleties of this kind may not be always picked up during interviews or in subsequent analyses. Furthermore, the success of interviews may be enhanced by the quality and skills of the translators or other intermediaries (see Materials and methods), but the need for translation nonetheless represents an additional step (between experience and "knowledge") and is a possible source of imprecision in the knowledge retrieved. The selected set of fishermen interviewed may also be a source of bias in the quality of the knowledge provided (Davis and Wagner 2003; Silvano and Begossi 2005), given the diverse status, specialization, and experience (Johannes et al. 2000; Grant and Berkes 2007) of the fishermen. We tried to minimize the impact of this bias by working with a broad range of individuals in various locations and then compiling the results obtained. However, it is difficult to determine whether working with another set of fishermen would have generated similar results. An example was provided by the study of species diets; overall, fishermen did not report the partly benthophagic diet of *Drepane africana*, whereas stomach analyses confirmed this diet, also reported by Baran (1995) and Diouf (1996) in Senegal. Another set of fishermen might have mentioned this feeding behavior. The use of a sampling saturation strategy (Davis and Wagner 2003), in which increasing numbers of fishermen are interviewed until no new substantial information is obtained, might help to overcome problems relating to this type of inaccuracy. However, this would require a much greater effort (Davis and Wagner 2003). Moreover, in this study we explored a wide range of subjects within various interview situations, such as the diversity of the periods in which meetings occurred, seasons, authority relationships within the groups interviewed, and the fishermen's personalities and their feelings about the survey (Ames 2007). This context generates an undetermined level of variability. These sources of inaccuracy may be difficult to avoid, making it

Fig. 7. Comparison of the trophic network reconstituted on the basis of (*a*) local ecological knowledge and (*b*) an Ecopath modeling approach (Guénette and Diallo 2004*a*, 2004*b*) for the species described by the fishermen. The thickness of the arrows in network *b* is proportional to the contribution of the relationship to the diet of the predator. The *y* axis on the right is the reported scale of the alternative figure for sake of comparison; the horizontal scale is arbitrary.


(a)
Fishermen 'trophic' level
V
IV
III
II
I
0
Corresponding EcoPath model trophic level (from b)
4
3
2
1
large size coastal Elasmobranchii /squales (sereki)
Arius sp. (konkoe)
medium size coastal Elasmobranchii, rhinobatids / rays (manteki)
Polydactylus quadrifilis (sori)
Pentanemus quinquarius (gbalakhassa)
Ethmalosa fimbriata bonga shad (bonga)
crustaceans / shrimps (sanfu)
detritus / plant debris (bourêkhê khonkhouri)
benthos / silt (bora)
scientific term / common name (soso term)
(b)
EcoPath model trophic level
4
3
2
1
Corresponding fishermen 'trophic' level (from a)
V
IV
III
II
I
0
medium size coastal Elasmobranchii rhinobatids (manteki)
large size coastal Elasmobranchii /squales (sereki)
Arius sp. catfish (konkoe)
Pentanemus quinquarius (gbalakhassa)
Polydactilus quadrifilis (sori)
Ethmalosa fimbriata bonga shad (bonga)
crustaceans / shrimps (sanfu)
benthos / silt (bora)
detritus / plant debris (bourêkhê khonkhouri)

harder to demonstrate quantified validity. Hence, in most cases, the domain of validity of the knowledge obtained was difficult to assess and the use of a qualitative "level of confidence" for each type of knowledge might give a more accurate evaluation of LEK

Based on the observations made within this study and in previous work, we identified two factors associated with a higher level of confidence in a given piece of information. First, knowledge directly related to fishing success appeared to be more reliable, because the acquisition of such knowl-

Table 3. Qualitative summary of the comparison between local ecological knowledge (LEK) and scientific knowledge for the topics studied.

	Ecology			Fish reproduction		Trophic relationships	
LEK compared with scientific knowledge	Ecological segmentation of the year	Seabed habitats	Edaphic preferences – biotopes	Nursery localization	Reproductive cycle	Fish diets	Trophic network
Overall agreement	–	+	+	–	–	+	+
Overall discrepancy	–	–	–	–	+	–	–
Only tractable by means of LEK	–	–	–	+	–	–	–
Provides complementary insight	+	–	–	–	–	–	–

Note: Four main situations can be identified that were (+) or were not (–) observed within the study.

edge is essential for successful fishing practice (Symes 2008). For example, the fishermen had extensive knowledge of each type of seabed, because this knowledge is an essential determinant of the type of fishing gear to be used and provides information about the type of resource present. Knowledge associated with repeated observations was also considered to increase the level of confidence (Williams and Bax 2007). Repeatability may be established through the existence of a large number of accounts of the same phenomenon, such as the identification of seasons, or may relate to recurrent practices. This is the case for the continuous comparison of catches with habitats that provides fishermen with knowledge of the preferences of the different groups of fish or the identification of diets through the repeated gutting of fish during the processing of catches. In this study, all the results presented correspond to knowledge amassed from repeated observations.

The systematic comparison of LEK with scientific knowledge gave an estimate of the reliability of LEK, particularly concerning the possible use of LEK as a knowledge source within an EAF. Several different situations were observed (Table 3).

An overall comparison was possible for most of the issues studied, but with differences in the degree of agreement obtained. Knowledge about fish diets appeared to be a straightforward case, with the possibility of an almost quantitative comparison (more than 80% concordance was observed between the two sources). It should be stressed that diet is highly variable and dependent on food availability and that much larger sample sizes would have been required to describe the diet of a particular species accurately. Nevertheless, discrepancies were found between the two data sources. On the one hand, the benthic diet of *Drepane africana* was confirmed by three scientific sources but not reported by fishermen. On the other hand, mollusks and crabs were not found in the diet of *Arius* sp., whereas fishermen were well aware of the opportunistic omnivorous nature of the Ariidae family (analyses showed the presence of miscellaneous wood and plastic objects in the stomachs of these voracious sea catfish).

Overall comparison was partly successful for species biotopes. In this case, differences can be observed in the knowledge recorded between different published studies, between published studies and the traditional knowledge of fishermen, and between phases I and II. When differences are identified between the sources of knowledge, we have no appropriate criteria for determining which sources are the most reliable or a threshold separating reliable and unreliable sources, because the observed habitat differences may be due to the great ecophysiological, reproductive, and dietary plasticity of these taxonomic groups (Baran 1995).

The information for seabed habitats was found to be concordant after reclassification of the seabed types identified by fishermen and in scientific approaches. This brings us back to the general agreement (Aswani and Lauer 2006; Ames 2007; Williams and Bax 2007) that LEK constitutes a rewarding source of knowledge for issues relating to seabed mapping. In the present case, LEK provided greater local detail than scientific survey of Domain and Bah (1993), which was constrained by low-resolution sampling; the scientific classification was established for the broad scale of the Guinean continental shelf, and the area studied is only part of this larger region. Other differences may be linked to differences in the intensity of sampling of the two approaches; only three samples were obtained in this area during the oceanographic transects leading to the construction of the scientific map. In this case, in accordance with the work of Huntington et al. (2004), these two results may be considered to provide complementary knowledge at two different scales.

Conversely, the comparison of the trophic networks obtained revealed that the Ecopath approach provided more details than LEK for trophic networks, because of inclusion of the relative strengths of the trophic relationships identified. Within the comparison, several other methodological features also differ between the two sources. The relationships described by the fishermen concerned the food actually ingested, whereas the Ecopath trophic level is an indirect indicator based on estimates of biomass transfer between levels (Christensen and Walters 2004; Gascuel 2005). As for seabed description, the LEK observation scale also differed from that used in scientific studies; Guénette and Diallo (2004*a*, 2004*b*) studied the entire EEZ, whereas fishermen described different local networks, sometimes even between estuaries. Differences in results were also found between the fishermen's perceptions and the modeling results. For example, the fishermen did not mention the importance of the benthos–debris relationship or the contribution of benthos. Self-predation relationships were also not recognized (or at least expressed) by fishermen. In the Ecopath model, the trophic relationships of the bonga shad were reduced exclusively to benthos consumption. By contrast, the fishermen considered this species to be a potential predator, particularly of shrimps. *Pentanemus quinquarius* was also identified as a high-level predator within the Ecopath model, whereas the fishermen considered it to be chiefly preyed upon. The fishermen also considered distinct "trophic levels" for the two types of Elasmobranchii: top level V for sharks and level III for smaller Rhinobatids. In all these cases, only partial agreement was obtained. However, the two sources appeared to give similar levels of knowledge with a certain number of major traits in common, including the diverse diet of Elasmobranchii, the predominance of shrimp as the principal food source in this food web, and the diversity of relationships between groups, most of which were identical for both approaches. The indicated differences did not reflect discrepancies in knowledge. Instead, they mostly reflected local conditions or the complementary nature of scientific and fishermen's knowledge.

In other cases, such as for the reproductive cycle of the bonga shad, discrepancies were found among the various sources of knowledge. There is a wide variability of the reproduction parameters described for this species (Charles-Dominique 1982), and differences have been observed between parts of a given estuary (Baran 1995). We therefore could not determine which piece of knowledge was valid. On the one hand, according to the approach of Maurstad et al. (2007), the descriptions of the reproductive cycle provided by fishermen for this particular species appear to be reliable, detailed narratives. These descriptions are also strongly supported by the extensive experience of the oldest fishermen concerning fish reproduction (Silvano et al. 2006). On the other hand the results presented by Baran (1995) were obtained by thorough analysis of bimestrial net sampling over a 1-year period. Such situations, with conflicting results, have been previously described (Johannes et al. 2000; Maurstad et al. 2007; Silvano and Begossi 2010). Johannes and Neis (2007) in particular reported a similar situation of wide discrepancies concerning the reproduction of barramundi (*Lates calcarifer*), for which further studies confirmed both sources of knowledge. However, in our case, no complementary study was available, and it was not possible to identify the threshold between accurate and incorrect knowledge.

In yet other situations, it was difficult to acquire knowledge or information through scientific studies. Indeed, the continuous activity and ubiquitous distribution of small-scale fishermen along the coastline results in a collective observation force (Williams and Bax 2007), well in excess of the likely provisions of an equivalent scientific protocol, in terms of both frequency and scope (Bergmann et al. 2007; García-Allut et al. 2007; Rochet et al. 2008). Repeatability greatly strengthens the contribution of LEK to EAF. This was the case for nursery localization; the identification of nurseries by scientific methods would require expensive detailed monitoring of multiple areas over a long period of time (Poizat and Baran 1997). However, fishermen were able, collectively, to develop maps of nursery locations including specific details for each estuary, and this knowledge may be crucial in the establishment of protected areas, for example, particularly for determining their location and size, to maximize conservation, biodiversity, and fishery benefits (Kupschus 2003; Browman and Stergiou 2004). Hence, this unique possibility to downscale argues for collaboration between scientists and fishermen in the design of future surveys.

The diverse set of standpoints (Table 3) reflects the diversity of contexts and issues linked to the use of LEK: substitutable, conflicting with its scientific equivalent, or with no scientific equivalent. Hence, there seems to be no set of common rules for the use of LEK, but different situations indicate that LEK should be used on a case-by-case basis, in each specific field of knowledge. This diversity of situations has been described before (Silvano and Valbo-Jørgensen 2008) and leads to different likelihood estimates of LEK. Based on the results of this study, for example, LEK could complement scientific studies (seabed description), be used as a source of new scientific investigation (bonga reproductive cycle), be considered as the only source of information (nursery location), could substitute for scientific surveys provided that the level of validity is identical (fish diets), or constitute a satisfactory proxy (trophic web).

Beyond addressing specific questions, LEK, as a single source of information, seems to address all the different dimensions (ecology, fish reproduction, trophic relationships) of ecosystem functioning simultaneously. The ecological segmentation of the year described by fishermen illustrates this well, with fishermen classifying the seasons more on the basis of ecological and local conditions than according to universal (spring–summer–autumn–winter) or regional (dry season – wet season) "climatic" classifications. Indeed, fishermen identified a set of criteria directly related to the seasonal clocks of the resources exploited (movements, abundances, arrivals, departures, replacements of taxonomic groups, weather). This indicates that they make use of a coherent synthesis of all the elements in a single scheme. This holistic approach has been reported before, in more general (Berkes et al. 2007; Symes 2008) or specific situations, such as the description of water characteristics (Barthélémy 2005) or traditional ecosystem resource knowledge and management (Poepoe et al. 2007). Going beyond case-by-case use, the fishermen's knowledge could therefore be considered as providing a complete functional description of the local ecosystem exploited. The differences in classification, insight, and viewpoints with respect to scientific studies (Moller et al. 2004; Berkes et al. 2007; this study) could indeed result in a complementary fishermen's "theory", paradigm (Ames 2007), or cosmology (Houde 2007) of marine resources and environment. This globally different insight could then constitute a complementary grid for ecosystem analysis, and understanding that it would be worthwhile to characterize further.

In developing countries lacking data and resources, such as the republic of Guinea, several authors have highlighted the practical relevance of LEK for obtaining knowledge (Johannes 1998; Silvano and Begossi 2010), particularly in the context of operational EAF, in which the cost of obtaining knowledge is an important argument (Cury et al. 2005; Garcia and Cochrane 2005). LEK could help to provide answers to questions relating to the identification of sensitive areas in terms of ecosystem productivity (Aswani and Hamilton 2004; Aswani and Lauer 2006), for which diverse knowledge along the entire coast must be obtained. The use of LEK may therefore be worth considering, not so much to guide management actions, although this use is possible (Silvano and Begossi 2010), but as a source of critical knowledge in areas in which it costs too much to carry out scientific studies (Maurstad et al. 2007). It would be possible to implement this approach by intensifying the two-way links between researchers and other actors, involving both mutual information acquisition and bidirectional structures (links) for communicating existing knowledge (Le Fur et al. 2002).

Acknowledgements

This work was supported by European Commission Contract B7-6200-99/03-DG DEV. We thank Robert Manet Karemba and Abdulaye "Sökhö" Camara (intermediaries), the local fishermen from Guinea who took part in this study, Djibril Wamounou Camara and Aïssatou Diallo for archive reconstruction, and Joëlle Vincent for assistance with fish drawings. We thank Marie-Joëlle Rochet, Denis Bourguet, and Serge Garcia for fruitful discussions and Nigel Haggan and Renato Silvano for substantial improvement of the manuscript. This work is dedicated to the memory of Athanase Guilavogui, who died during the writing of this article.

References

Ames, T. 2007. Putting fisher's knowledge to work: reconstructing the Gulf of Maine cod spawning grounds on the basis of local ecological knowledge. *In* Fishers' knowledge in fisheries science and management. Coastal Management Sourcebooks, Vol. 4. *Edited by* N. Haggan, B. Neis, and I.G. Baird. UNESCO Publishing, Paris. pp. 353–364.

Aswani, S., and Hamilton, R. 2004. Integrating indigenous ecological knowledge and customary sea tenure with marine and social science for conservation of bumphead parrotfish (*Bolbometopon muricatum*) in the Roviana Lagoon, Solomon Islands. Environ. Conserv. **31**(1): 69–83. doi:10.1017/S037689290400116X.

Aswani, S., and Lauer, M. 2006. Benthic mapping using local aerial photo interpretation and resident taxa inventories for designing marine protected areas. Environ. Conserv. **33**(3): 263–273. doi:10.1017/S0376892906003183.

Baelde, P. 2007. Using fisher's knowledge goes beyond filling gaps in scientific knowledge: analysis of Australian experiences. *In* Fishers' knowledge in fisheries science and management. Coastal Management Sourcebooks, Vol. 4. *Edited by* N. Haggan, B. Neis, and I.G. Baird. UNESCO Publishing, Paris. pp. 381–400.

Baran, E. 1995. Dynamique spatio-temporelle des peuplements de poissons estuariens en Guinée: relations avec le milieu abiotique. Ph.D. thesis, Travaux et Documents Microédités, Paris, France.

Baran, E. 2000. Rôle des estuaires vis à vis de la ressource halieutique côtière en Guinée. *In* La pêche côtière en Guinée: ressources et exploitation. *Edited by* F. Domain, P. Chavance, and A. Diallo. IRD Ed., Paris, France. pp. 145–147.

Barthélémy, C. 2005. Les savoirs locaux: entre connaissances et reconnaissance. Vertig0 — La Revue En Sciences de L'Environnement, **6**(1): 1–6.

Bergmann, M., Hinz, B., Blyth, R., Kaiser, M.J., Rogers, S.I., and Armstrong, M. 2007. Using expert knowledge to identify possible groundfish 'essential fish habitats'. *In* Fishers' knowledge in fisheries science and management. Coastal Management Sourcebooks, Vol. 4. *Edited by* N. Haggan, B. Neis, and I.G. Baird. UNESCO Publishing, Paris. pp. 189–197.

Berkes, F. 2008. Sacred ecology. 2nd ed. Routledge, New York.

Berkes, F., Berkes, M.K., and Fast, H. 2007. Collaborative integrated management in Canada's North: the role of local and traditional knowledge and community-based monitoring. Coast. Manage. **35**(1): 143–162. doi:10.1080/08920750600970487.

Browman, H.I., and Stergiou, K.I. 2004. Perspectives on ecosystem-based approaches to the management of marine resources. Mar. Ecol. Prog. Ser. **274**: 269–303. doi:10.3354/meps274269.

Charles-Dominique, E. 1982. Exposé synoptique des données biologiques sur l'ethmalose (*Ethmalosa fimbriata* S. Bowdich, 1825. Rev. Hydrobiol. Trop. **15**(4): 373–397.

Chavance, P. 2000. Trait caractéristiques et évolution récente de la pêche artisanale. *In* La pêche côtière en Guinée: ressources et exploitation. *Edited by* F. Domain, P. Chavance, and A. Diallo. IRD Ed., Paris, France. pp. 295–311.

Christensen, V., and Pauly, D. 1992. Ecopath II — a software for balancing steady-state ecosystem models and calculating network characteristics. Ecol. Model. **61**(3–4): 169–185. doi:10.1016/0304-3800(92)90016-8.

Christensen, V., and Walters, C.J. 2004. Ecopath with Ecosim: methods, capabilities and limitations. Ecol. Model. **172**(2–4): 109–139. doi:10.1016/j.ecolmodel.2003.09.003.

Christie, P., Fluharty, D.L., White, A.T., Eisma-Osorio, L., and Jatulan, W. 2007. Assessing the feasibility of ecosystem-based fisheries management in tropical contexts. Mar. Policy, **31**(3): 239–250. doi:10.1016/j.marpol.2006.08.001.

Cury, P., Shannon, L.J., Roux, J.-P., Daskalov, G.M., Jarre, A., Moloney, C.L., and Pauly, D. 2005. Trophodynamic indicators for an ecosystem approach to fisheries. ICES J. Mar. Sci. **62**(3): 430–442. doi:10.1016/j.icesjms.2004.12.006.

D'Incao, F., and Reis, E.G. 2002. Community-based management and technical advice in Patos Lagoon estuary (Brazil). Ocean Coast. Manage. **45**(8): 531–539. doi:10.1016/S0964-5691(02)00084-4.

Davis, A., and Wagner, J.R. 2003. Who knows? On the importance of identifying "experts" when researching local ecological knowledge. Hum. Ecol. **31**(3): 463–489. doi:10.1023/A:1025075923297.

Davis, A., Hanson, J.M., Watts, H., and MacPherson, H. 2004. Local ecological knowledge and marine fisheries research: the case of white hake (*Urophycis tenuis*) predation on juvenile American lobster (*Homarus americanus*). Can. J. Fish. Aquat. Sci. **61**(7): 1191–1201. doi:10.1139/f04-070.

Diouf, P.S. 1996. Les peuplements de poissons des milieux estuariens de l'Afrique de l'Ouest: l'exemple de l'estuaire hyperhalin du Sine-Saloum. Ph.D. thesis, Université Montpellier II, Paris, France.

Domain, F., and Bah, M.O. 1993. Carte sédimentologique du plateau continental guinéen. Orstom, Paris, France. [Notice explicative: 108, p. 15, 2 maps.]

Domain, F., Keita, M., and Morize, E. 2000. Typologie générale des ressources démersales du plateau continental. *In* La pêche côtière en Guinée: ressources et exploitation. *Edited by* F. Domain, P. Chavance, and A. Diallo. IRD Ed., Paris, France. pp. 53–85.

Fletcher, W.J. 2006. Frameworks for managing marine resources in Australia through ecosystem approaches: Do they fit together and are they useful? Bull. Mar. Sci. **78**(3): 691–704.

Folke, C. 2004. Traditional knowledge in social–ecological systems. Ecol. Soc. 9(3): 7. Available from http://www.ecologyandsociety.org/vol9/iss3/art7/.

Garcia, S.M., and Cochrane, K.L. 2005. Ecosystem approach to fisheries: a review of implementation guidelines. ICES J. Mar. Sci. **62**(3): 311–318. doi:10.1016/j.icesjms.2004.12.003.

Garcia, S.M., Zerbi, A., Aliaume, C., Do Chi, T., and Lasserre, G. 2003. The ecosystem approach to fisheries. Issues, terminology, principles, institutional foundations, implementation and outlook. FAO Fish. Tech. Pap. No. 443. Rome, Italy.

García-Allut, A., Freire, J., Barreiro, A., and Losada, D.E. 2007. Methodology for integration of fisher's ecological knowledge in fisheries biology and management using knowledge representation (artificial intelligence). *In* Fishers' knowledge in fisheries science and management. Coastal Management Sourcebooks, Vol. 4. *Edited by* N. Haggan, B. Neis, and I.G. Baird. UNESCO Publishing, Paris. pp. 227–237.

Gascuel, D. 2005. The trophic-level based model: a theoretical approach of fishing effects on marine ecosystems. Ecol. Model. **189**(3–4): 315–332. doi:10.1016/j.ecolmodel.2005.03.019.

Gascuel, D., Guénette, S., and Pauly, D. 2008. The trophic-level based ecosystem modelling approach: theoretical overview and practical uses. ASC-ICES CM 2008 / F:18. Halifax, N.S., Canada.

Gerhardinger, L.C., Godoy, E.A.S., and Jones, P.J.S. 2009. Local ecological knowledge and the management of marine protected areas in Brazil. Ocean Coast. Manage. **52**(3–4): 154–165. doi:10.1016/j.ocecoaman.2008.12.007.

Grant, S., and Berkes, F. 2007. Fisher knowledge as expert system: a case from the longline fishery of Grenada, the eastern Caribbean. Fish. Res. **84**: 162–170.

Gray, T., and Hatchard, J. 2008. A complicated relationship: stakeholder participation and the ecosystem-based approach to fisheries management. Mar. Policy, **32**(2): 158–168. doi:10.1016/j.marpol.2007.09.002.

Greenstreet, S.P.R., Fraser, H.M., and Piet, G.J. 2009. Using MPAs to address regional-scale ecological objectives in the North Sea: modelling the effects of fishing effort displacement. ICES J. Mar. Sci. **66**(1): 90–100. doi:10.1093/icesjms/fsn214.

Guénette, S., and Diallo, I. 2004*a*. Modèles préliminaires de la côte guinéenne pour les années 1985 et 1998. *In* Northwest African marine ecosystems. *Edited by* M.L.D. Palomares, J.M. Vakily, and D. Pauly. Fisheries Centre Research Report, UBC, Vancouver, B. C. Vol. 12(7). pp. 124–159.

Guénette, S., and Diallo, I. 2004*b*. Exploration of a preliminary model of the marine ecosystem of Guinea. *In* International Symposium on Marine Fisheries, Ecosystems, and Societies in West Africa: Half a Century of Change, Dakar, Senegal, 24–28 June 2002. *Edited by* P. Chavance, M. Bâ, D. Gascuel, J.M. Vakily, and D. Pauly. Office of Official Publications of the European Commission, Bruxelles, Collection des Rapports de recherche Halieutique. ACP-UE. Vol. 15(1): 329–346.

Guilavogui, A., Le Fur, J., and Doumbouya, A. 2004. Accès à l'espace et à la ressource: compétitions et conflits entre la pêche artisanale et la pêche industrielle dans la ZEE guinéenne. Doc. Sci. Centr. Nat. Sci. Halieut. No. 36. Boussoura, Conakry, République de Guinée.

Gullström, M., Lunden, B., Bodin, M., Kangwe, J., Öhman, M., Mtolera, M.S.P., and Björk, M. 2006. Assessment of changes in the seagrass-dominated submerged vegetation of tropical Chwaka Bay (Zanzibar) using satellite remote sensing. Estuar. Coast. Shelf Sci. **67**(3): 399–408. doi:10.1016/j.ecss.2005.11.020.

Haggan, N., and Neis, B. 2007. The changing face of fisheries science and management. *In* Fishers' knowledge in fisheries science and management. Coastal Management Sourcebooks, Vol. 4. *Edited by* N. Haggan, B. Neis, and I.G. Baird. UNESCO Publishing, Paris. pp. 421–432.

Hall, G.B., Moore, A., Knight, P., and Hankey, N. 2009. The extraction and utilization of local and scientific geospatial knowledge within the Bluff oyster fishery, New Zealand. J. Environ. Manage. **90**(6): 2055–2070. doi:10.1016/j.jenvman.2007.08.022. PMID:18760525.

Heck, S., Béné, C., and Reyes-Gaskin, R. 2007. Investing in African fisheries: building links to the Millennium Development Goals. Fish Fish. **8**: 211–226.

Hickey, F.R. 2007. Traditional marine resource management in Vanuatu: world views in transformation. *In* Fishers' knowledge in fisheries science and management. Coastal Management Sourcebooks, Vol. 4. *Edited by* N. Haggan, B. Neis, and I.G. Baird. UNESCO Publishing, Paris. pp. 147–168.

Houde, N. 2007. The six faces of traditional ecological knowledge: challenges and opportunities for Canadian co-management arrangements. Ecol. Soc. 12(2): 34. Available from http://www.ecologyandsociety.org/vol12/iss2/art34/.

Huntington, H.P., Suydam, R.S., and Rosenberg, D.H. 2004. Traditional knowledge and satellite tracking as complementary approaches to ecological understanding. Environ. Conserv. **31**(3): 177–180. doi:10.1017/S0376892904001559.

International Union for Conservation of Nature. 2007. La Guinée, soucieuse de la préservation environnementale: des partenaires dynamiques dans un contexte difficile. Lettre d'information PRCM (Programme Régional de Conservation de la Zone Côtière et Marine en Afrique de l'Ouest), Nouakchott, Mauritanie, mars 2007. Available from http://www.prcmarine.org/wrap/newsletters/2007/03.pdf [accessed 4 March 2011].

International Union for Conservation of Nature. 2008. Evaluation des aires protégées de Guinée (février 2008). La lettre des aires protégées en Afrique de l'Ouest, IUCN, Ouagadougou, Burkina Faso, avril 2008(7): 8. Available from http://papaco.org/publication/lettreAPAO-7-0408.pdf [accessed 4 March 2011].

Johannes, R.E. 1981. Working with fishermen to improve coastal tropical fisheries and resource management. Bull. Mar. Sci. **31**(3): 673–680.

Johannes, R.E. 1984. Marine conservation in relation to traditional lifestyles of tropical artisanal fishermen. Environmentalist, **4**(Suppl. 7): 30–35. doi:10.1007/BF01907290.

Johannes, R.E. 1998. The case for data-less marine resource management: examples from tropical nearshore finfisheries. Trends Ecol. Evol. **13**(6): 243–246. doi:10.1016/S0169-5347(98)01384-6. PMID:21238285.

Johannes, R.E., and Neis, B. 2007. The value of anecdote. *In* Fishers' knowledge in fisheries science and management. Coastal Management Sourcebooks, Vol. 4. *Edited by* N. Haggan, B. Neis, and I. G. Baird. UNESCO Publishing, Paris. pp. 41–58.

Johannes, R.E., Freeman, M.M.R., and Hamilton, R.J. 2000. Ignore fishers' knowledge and miss the boat. Fish Fish. **1**(3): 257–271.

Kupschus, S. 2003. Development and evaluation of statistical habitat suitability models: an example based on juvenile spotted seatrout *Cynoscion nebulosus*. Mar. Ecol. Prog. Ser. **265**: 197–212. doi:10.3354/meps265197.

Le Fur, J., Doumbouya, A., Diallo, M.M., Camara, Y., and Domain, F. 2002. Construction et mise à disposition par la recherche d'un savoir commun: enjeux et application au secteur des pêches guinéen. *In* Compte-Rendu du séminaire de travail: La recherche halieutique et le développement durable des ressources naturelles marines de l'Afrique de l'Ouest: quels enjeux? Conakry, Guinée, 24–26 septembre 2001. *Edited by* P. Failler, M. Bâ, A. Doumbouya, and N. Lécrivain. Bruxelles, Rapp. Rech. Halieut. ACP-UE, 11: 110–115.

Lesnoff, M., Morize, E., and Traore, S. 2000. La pêcherie industrielle en Guinée: état et bilan des données disponibles. *In* La pêche côtière en Guinée: ressources et exploitation. *Edited by* F. Domain, P. Chavance, and A. Diallo. IRD Ed., Paris. pp. 175–198.

Longhurst, A.R. 1966. Species Assemblages in the Tropical Demersal Fisheries. Proceedings of the Symposium on Oceanography and Fisheries Resources of Tropical Atlantic, Abidjan, 20–28 October 1966. UNESCO, Paris. pp. 147–170.

Marasco, R.J., Goodman, D., Grimes, C.B., Lawson, P., Punt, A.E., and Quinn, T.J., II. 2007. Ecosystem-based fisheries management: some practical suggestions. Can. J. Fish. Aquat. Sci. **64**(6): 928–939. doi:10.1139/f07-062.

Mathew, S. 2003. Small-scale fisheries perspectives on an ecosystem-based approach to fisheries management. *In* Responsible fisheries in the marine ecosystem. *Edited by* M. Sinclair and G. Valdimarsson. Cabi Publishing, Wallingford, UK. pp. 47–63. [ISBN 0851996337]

Maurstad, A., Dale, T., and Bjørn, P.A. 2007. You wouldn't spawn in a septic tank, would you? Hum. Ecol. **35**(5): 601–610. doi:10.1007/s10745-007-9126-5.

Moller, H., Berkes, F., O'Brian Lyver, P., and Kislalioglu, M. 2004.

Combining science and traditional ecological knowledge: monitoring populations for co-management. Ecol. Soc. 9(3): 2. Available from http://www.ecologyandsociety.org/vol9/iss3/art2/.

Moreno, G., Dagorn, L., Sancho, G., and Itano, D. 2007. Fish behaviour from fisher's knowledge: the case study of tropical tuna around drifting fish aggregating devices (DFADs). Can. J. Fish. Aquat. Sci. **64**(11): 1517–1528. doi:10.1139/f07-113.

Neis, B., Schneider, D.C., Felt, L., Haedrich, R.L., Fischer, J., and Hutchings, J.A. 1999. Fisheries assessment: what can be learned from interviewing resource users? Can. J. Fish. Aquat. Sci. **56**(10): 1949–1963. doi:10.1139/f99-115.

Olsson, P., and Folke, C. 2001. Local ecological knowledge and institutional dynamics for ecosystem management: a study of Lake Racken Watershed, Sweden. Ecosystems, **4**(2): 85–104. doi:10.1007/s100210000061.

Paterson, B., and Petersen, S.L. 2010. EAF implementation in Southern Africa: lessons learnt. Mar. Policy, **34**(2): 276–292. doi:10.1016/j.marpol.2009.07.004.

Pezennec, O. 2000. Environnement hydro-climatique de la Guinée. *In* La pêche côtière en Guinée: ressources et exploitation. *Edited by* F. Domain, P. Chavance, and A. Diallo. IRD Ed., Paris, France. pp. 7–27.

Pikitch, E.K., Santora, C., Babcock, E.A., Bakun, A., Bonfil, R., Conover, D.O., Dayton, P., Doukakis, P., Fluharty, D., Heneman, B., Houde, E.D., Link, J., Livingston, P.A., Mangel, M., McAllister, M.K., Pope, J., and Sainsbury, K.J. 2004. Ecosystem-based fishery management. Science, **305**(5682): 346–347. doi:10.1126/science.1098222. PMID:15256658.

Pitcher, T.J., Kalikoski, D., Short, K., Varkey, D., and Pramod, G. 2009. An evaluation of progress in implementing ecosystem-based management of fisheries in 33 countries. Mar. Policy, **33**(2): 223–232. doi:10.1016/j.marpol.2008.06.002.

Poepoe, K.K., Bartram, P.K., and Friedlander, A.M. 2007. The use of traditional knowledge in the contemporary management of a Hawaiian community's marine resources. *In* Fishers' knowledge in fisheries science and management. Coastal Management Sourcebooks, Vol. 4. *Edited by* N. Haggan, B. Neis, and I.G. Baird. UNESCO Publishing, Paris. pp. 119–143.

Poizat, G., and Baran, E. 1997. Fishermen's knowledge as background information in tropical fish ecology: a quantitative comparison with fish sampling results. Environ. Biol. Fishes, **50**(4): 435–449. doi:10.1023/A:1007317423165.

Prigent, M., Fontenelle, G., Rochet, M.J., and Trenkel, V.M. 2008. Using cognitive maps to investigate fishers' ecosystem objectives and knowledge. Ocean Coast. Manage. **51**(6): 450–462. doi:10.1016/j.ocecoaman.2008.04.005.

Rice, J. 2008. Can we manage ecosystems in a sustainable way? J. Sea Res. **60**(1–2): 8–20. doi:10.1016/j.seares.2008.02.002.

Rochet, M.J., Prigent, M., Bertrand, J.A., Carpentier, A., Coppin, F., Delpech, J.P., Fontenelle, G., Foucher, E., Mahé, K., Rostiaux, E., and Trenkel, V.M. 2008. Ecosystem trends: evidence for agreement between fishers' perceptions and scientific information. ICES J. Mar. Sci. **65**(6): 1057–1068. doi:10.1093/icesjms/fsn062.

Saenger, P., and Hegerl, E.J. 1983. Global status of mangrove ecosystems. Environmentalist, **3**(Suppl. 3): 1–88.

Schneider, W. 1990. FAO species identification sheets for fishery purposes. Field guide to the commercial marine resources of the Gulf of Guinea. FAO, Rome, RAFR/FI/90/2.

Seto, K.C., and Fragkias, M. 2007. Mangrove conversion and aquaculture development in Vietnam: a remote sensing-based approach for evaluating the Ramsar Convention on Wetlands. Glob. Environ. Change, **17**(3–4): 486–500. doi:10.1016/j.gloenvcha.2007.03.001.

Sherman, K., and Duda, A.M. 1999. An ecosystem approach to global assessment and management of coastal waters. Mar. Ecol. Prog. Ser. **190**: 271–287. doi:10.3354/meps190271.

Sidibe, A. 2003. Les ressources halieutiques démersales côtières de la Guinée: exploitation, biologie et dynamique des principales espèces de la communauté à Sciaenidés. Ph.D. thesis, Ensa-Rennes, France.

Sidibe, A., Laurans, M., Gascuel, D., and Domain, F. 2004. Evolution comparative de l'abondance des ressources halieutiques démersales en Guinée entre 1985 et 1998. *In* Pêcheries maritimes, écosystèmes et sociétés en Afrique de l'Ouest: un demi siècle de changement. *Edited by* P. Chavance, M. Bâ, D. Gascuel, J.M. Vakily, and D. Pauly. Office des Communautés Européennes, Coll. Rapports de Recherche Halieutique ACP-UE 15, Luxembourg. pp. 393–398.

Silvano, R.A.M., and Begossi, A. 2005. Local knowledge on a cosmopolitan fish ethnoecology of *Pomatomus saltatrix* (Pomatomidae) in Brazil and Australia. Fish. Res. **71**(1): 43–59. doi:10.1016/j.fishres.2004.07.007.

Silvano, R.A.M., and Begossi, A. 2010. What can be learned from fishers? An integrated survey of fisher's local ecological knowledge and bluefish (*Pomatomus saltatrix*) biology on the Brazilian coast. Hydrobiologia, **637**(1): 3–18. doi:10.1007/s10750-009-9979-2.

Silvano, R.A.M., and Valbo-Jørgensen, J. 2008. Beyond fishermen's tales: contributions of fishers' local ecological knowledge to fish ecology and fisheries management. Environ. Dev. Sustain. **10**(5): 657–675. doi:10.1007/s10668-008-9149-0.

Silvano, R.A.M., Udvardy, S., Ceroni, M., and Farley, J. 2005. An ecological integrity assessment of a Brazilian Atlantic Forest watershed based on surveys of stream health and local farmers' perceptions: implications for management. Ecol. Econ. **53**(3): 369–385. doi:10.1016/j.ecolecon.2004.12.003.

Silvano, R.A.M., MacCord, P.F.L., Lima, R.V., and Begossi, A. 2006. When does this fish spawn? Fishermen's local knowledge of migration and reproduction of Brazilian coastal fishes. Environ. Biol. Fishes, **76**(2-4): 371–386. doi:10.1007/s10641-006-9043-2.

Sinclair, M., Arnason, R., Csirke, J., Karnicki, Z., Sigurjonsson, J., Rune Skjoldal, H., and Valdimarsson, G. 2002. Responsible fisheries in the marine ecosystem. Fish. Res. **58**(3): 255–265. doi:10.1016/S0165-7836(02)00168-6.

Sissenwine, M., and Murawski, S. 2004. Moving beyond 'intelligent-tinkering': advancing an ecosystem approach to fisheries. Mar. Ecol. Prog. Ser. **274**: 291–295.

Symes, D. 2008. Fishers' knowledge in fisheries science and management. [Book review.] Fish. Res. **89**: 309–310. doi:10.1016/j.fishres.2007.10.001.

Ukwe, C.N., Ibe, C.A., and Sherman, K. 2006. A sixteen-country mobilization for sustainable fisheries in the Guinea Current Large Marine Ecosystem. Ocean Coast. Manage. **49**(7–8): 385–412. doi:10.1016/j.ocecoaman.2006.04.006.

Williams, A., and Bax, N. 2007. Integrating fisher's knowledge with survey data to understand the structure, ecology and use of a seascape off south-eastern Australia. *In* Fishers' knowledge in fisheries science and management. Coastal Management Sourcebooks, Vol. 4. *Edited by* N. Haggan, B. Neis, and I.G. Baird. UNESCO Publishing, Paris. pp. 365–380.

Wilson, D.C., Raakjær, J., and Degnbol, P. 2006. Local ecological knowledge and practical fisheries management in the tropics: a policy brief. Mar. Policy, **30**(6): 794–801. doi:10.1016/j.marpol.2006.02.004.

Zhang, C.I., Kim, S., Gunderson, D., Marasco, R., Lee, J.B., Park, H.W., and Lee, J.H. 2009. An ecosystem-based fisheries assessment approach for Korean fisheries. Fish. Res. **100**(1): 26–41. doi:10.1016/j.fishres.2008.12.002.

Phenotypic plasticity in physiological status at emergence from nests as a response to temperature in Atlantic salmon (*Salmo salar*)

Helge Skoglund, Sigurd Einum, Torbjørn Forseth, and Bjørn Torgeir Barlaup

Abstract: Successful transitions from relying on yolk to exogenous feeding may be strongly influenced by temperature conditions experienced both during embryonic development, through effects on juvenile phenotype, and during initiation of feeding. Here we simultaneously assess these two effects of temperature treatments (2, 5, 8, and 12 °C) in Atlantic salmon (*Salmo salar*). Fry emerging from low incubation temperatures were smaller and had metabolized more energy prior to emergence, but had larger yolk sacs and higher mass specific energy levels, than those experiencing higher temperatures. After emergence, activity, feeding, and growth increased significantly with increasing temperature, but fry were able to initiate feeding and maintain positive growth at all four temperatures. Larger energy stores may provide an advantage when emerging at cold temperatures with a low potential for feeding activity, whereas having a large body size, which is primarily of importance in competitive interactions, may be less important owing to cryptic feeding and sheltering at low temperatures. However, the adaptive significance of the observed phenotypic response to incubation temperature remains untested.

Résumé : Le passage avec succès de la dépendance du vitellus à l'alimentation exogène peut être fortement influencé par les conditions de température subies durant le développement embryonnaire, à travers leurs effets sur le phénotype juvénile, ainsi que durant l'initiation de l'alimentation. Nous évaluons simultanément ici ces deux effets des traitements thermiques (2, 5, 8 et 12 °C) chez le saumon atlantique (*Salmo salar*). Les alevins qui émergent de températures d'incubation basses sont plus petits et ont métabolisé plus d'énergie avant l'émergence, mais ils possèdent des sacs vitellins plus gros et des niveaux d'énergie spécifiques à la masse plus grands que ceux qui ont connu des températures plus élevées. Après l'émergence, l'activité, l'alimentation et la croissance augmentent significativement en fonction de la température croissante; cependant, les alevins sont capables de commencer à s'alimenter et de maintenir une croissance positive aux quatre températures. Les réserves plus importantes d'énergie peuvent représenter un avantage lors d'une émergence à basse température lorsque le potentiel d'activité alimentaire est faible; en revanche, une forte taille corporelle, qui est de première importance dans les interactions de compétition, peut être moins avantageuse pour l'alimentation cryptique et la mise à l'abri aux températures basses. Cependant, la valeur adaptative de la réponse phénotypique observée à la température d'incubation reste à tester.

[Traduit par la Rédaction]

Introduction

The transition from relying on parentally provided resources to independent exogenous feeding represents a critical period of the life cycle in many organisms. Typically, a considerable amount of the lifetime mortality may occur during the short period following this transition (Einum and Fleming 2000; Nislow and King 2006). The environmental conditions during such critical periods may have strong effects on performance, and hence ultimately fitness and population dynamics (Crecco and Savoy 1985; Jensen and Johnsen 1999). Further, survival may be strongly influenced by traits such as body size and physiological condition (Einum and Fleming 2000; Berkeley et al. 2004). Determining how environmental factors affect offspring phenotypes, as well as the ability of offspring to perform across environmental gradients during such critical periods, is therefore important for understanding how environments can shape population recruitment.

In seasonal environments, the environmental conditions experienced during such critical life history shifts will be strongly influenced by the phenology or the seasonal timing of the transition. In fish, the match or mismatch with appropriate seasonal conditions is often considered to be one of the key drivers for determining recruitment variability (Cushing 1990) and one of the main mechanisms underlying the evolution of spawning times (reviewed by Wright and Trippel 2009). Temperature plays a crucial role here since it is a

Received 17 December 2010. Accepted 27 March 2011. Published at www.nrcresearchpress.com/cjfas on 23 August 2011.
J22166

H. Skoglund and B.T. Barlaup. Laboratory of Freshwater Ecology and Inland Fisheries, Uni Research, Thormøhlensgate 49, N-5006 Bergen, Norway.
S. Einum. Centre for Conservation Biology, Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway; Norwegian Institute for Nature Research, Trondheim, Norway.
T. Forseth. Norwegian Institute for Nature Research, Trondheim, Norway.

Corresponding author: H. Skoglund (e-mail: helge.skoglund@uni.no).

Can. J. Fish. Aquat. Sci. **68**: 1470–1479 (2011) doi:10.1139/F2011-056 Published by NRC Research Press

strong determinant of development rate and, together with spawning time, is the main factor determining the phenology of offspring. Whereas effects of temperature on development rate in fish embryos and larvae are well described (Kamler 1992), less is known about how temperature affects the ability of offspring to successfully initiate exogenous feeding.

Temperature may also influence the size and physiological condition of the individuals during the transition to exogenous feeding. Although traits such as body size and size of yolk reserves are mainly considered to be determined by maternal traits such as egg size, temperature may affect yolk conversion efficiency (Heming 1982; Ojanguren et al. 1999), and hence how available resources are allocated within offspring. Further, in organisms where the transition to exogenous feeding is associated with pronounced habitat shifts, there may be an additional behavioural component. Body size and condition will depend on the development state (or ontogeny) of offspring when the habitat shift is triggered, and the ontogenetic timing of this shift may be influenced by environmental factors (Jones et al. 2003; Vonesh and Warkentin 2006). A precocious transition will result in the offspring entering the new environment with a smaller body size but with a larger yolk reserve, whereas a delay in the transition will have the opposite effect.

Here we test how temperature affects both phenotype of juvenile Atlantic salmon (*Salmo salar*) emerging from nests and their transition to exogenous feeding. The Atlantic salmon spawns in autumn by burying the eggs within nests in the streambed gravel, where they incubate throughout the winter. The alevins (yolk-sac larvae) remain in the nest after hatching while utilizing energy from the maternally provided yolk sac. When the yolk supply is nearly depleted in spring or early summer, the fry emerge from the nest, initiate exogenous feeding, and establish and defend feeding territories. It is not known what triggers this emergence behaviour, although it seems clear that it should occur when the fry have reached a development stage when they are both physiologically and behaviourally capable of initiating feeding and exhibiting other appropriate behaviours (i.e., social interactions and predator avoidance). Fry may emerge with different amounts of yolk remaining (García De Leániz et al. 2000; Skoglund and Barlaup 2006). Although the physiological timing (i.e., level of development) as a response to temperature has not previously been studied, fry appear capable to adjust their timing in response to environmental factors such as chemical cues from predators (Jones et al. 2003). Furthermore, there is little empirical data on how temperature affects performance during initiation of feeding, especially at low temperatures. Growth models of older Atlantic salmon juveniles suggest that feeding usually ceases at temperatures below 5–6 °C (Forseth et al. 2001; Jonsson et al. 2001), suggesting that this represents a lower thermal limit for successful transitions. However, thermal performance relationships may depend upon season and life history stage (e.g., Finstad et al. 2004). Peterson and Martin-Robichaud (1989) performed growth studies during first feeding, but were not able to resolve whether feeding and growth occurred at temperatures below 8 °C. Here we test whether temperature during the terminal period of nest incubation affects the body size and physiological status of emerging fry from artificial nests, as well as how temperatures influence postemergence growth and activity patterns of fry during the transition to exogenous feeding.

Materials and methods

Experimental site and fish

The experiment was conducted at the Norwegian Institute for Nature Research (NINA) Research Station, Ims, southwestern Norway, during March–June 2007. On 6 December 2006, 13 females and 13 males were artificially stripped. All parental fish were 1-sea-winter fish of the River Imsa population that had originally been released as 1-year-old smolts from the research station and caught in a trap in the river during return migrations. After fertilization, the family groups (full sibs) were mixed, and the eggs and alevins were incubated in the hatchery at a mean temperature of 7.0 °C (range: 1.4–10.2 °C). Based on data from the hatchery, fry emergence from nests in this population is likely to occur in early to middle of May, when stream temperatures typically have reached about 10–14 °C (K. Pettersen, NINA Research Station, Ims, Norway, personal communication 2011).

Experimental design

One hundred alevins from the mixed group were transferred to each of 32 artificial nests (eight replicates at each of four different temperature treatments, Table 1) on 15 March 2007. At this time, the alevins had exhausted a large part of the yolk sac but were prior to emergence (mean wet mass ± SD: 94.7 ± 15.2 mg). The artificial nests consisted of 5 L plastic buckets containing ~15 cm of gravel. The gravel was covered with a polystyrene lid perforated with four exit tubes. This prevented the fry (newly emerged juveniles) from re-entering the gravel after emergence and allowed them to be collected without disturbing the nest. A continuous water flow of 0.7 $L \cdot min^{-1}$ was provided through the nests by an inflow tube at the bottom of the bucket and an outlet through a meshed opening in the upper part of the bucket.

To test if the experimental design influenced time of emergence beyond the predicted temperature effects, the observed emergence times were compared with median emergence times predicted from a well-established empirically based development model for Atlantic salmon (Crisp 1981, 1988).

Water to all treatments was taken from Lake Liavatn, which is the headwater to the River Imsa and the water source for the research station. All treatments were kept under a constant light regime of 16 h L:8 h D. Temperatures were recorded on an hourly basis for each treatment. The nests were examined every morning for emerged fry, and 50% of the newly emerged fry from each nest were frozen for later analysis of body composition. The remaining fry were successively transferred to a growth experiment (see below).

To assess the effects of temperature on growth during first feeding, emerging fry were transferred to start feeding in tanks successively as they emerged from the nests. The tanks were 60 cm × 60 cm × 60 cm and had a water level of 20 cm, water flow of 1.5 $L \cdot min^{-1}$, and the same light regime as for the nests (16 h L:8 h D). The fry were fed in excess with commercial fish food (Ewos micro 0.15). To avoid large temporal asynchrony of fry with their introduction to tanks

Table 1. Mean and range of daily temperatures and duration of experimental period for different temperature groups during incubation and growth in tanks.

Incubation			Growth in tanks			
Temperature (°C)			Temperature (°C)			
Mean	Range	Period	Mean	Range	Period	No. of fry per tank
12.2	12.1–12.3	16 March–18 April	12.3	12.2–12.4	21 March–18 April	48 (43–53)
8.3	7.9–9.0	16 March–25 April	8.2	7.8–8.9	28 March–25 April	46 (43–49)
5.0	3.8–5.8	16 March–30 May	4.8	3.8–5.6	8 March–8 May	45.5 (39–50)
2.4	1.6–4.6	16 March–14 June	2.1	1.4–4.3	25 April–31 May	42 (33–48)

Note: Mean number of Atlantic salmon fry (max.–min.) introduced into each tank at each temperature treatment is also given.

and problems with managing feeding in tanks with few fish, both the first and last 5% of emerging fish from each nest were excluded from the tank experiments. Fry from each individual nest were transferred to one tank, providing eight replicate tanks for each temperature treatment. The total number of fry varied somewhat among tanks owing to variation in survival among nests (Table 1). For each temperature treatment, the growth experiment was run for 21 days from the time when 50% of the fry had emerged from the nests. The fish were then killed with an overdose of anaesthetic (MS-222) and frozen for later body composition analyses. In two of the tanks in the 8 °C treatment and three tanks in the 12 °C treatment, increased mortality was observed, possibly owing to bacterial infections or the parasitic flagellate *Ichthyobodo necator*. All fish showing signs of reduced health were removed from the tanks. To further reduce infection, all temperature treatments were treated with formalin solution (10 mL of a solution of 35% formalin and 15% methanol in 30 L water) for 10 min. The affected tanks were excluded from the survival analysis, but as the surviving fish in these tanks appeared not to be influenced in terms of growth, they were included in the growth analysis. Differences in densities in tanks did not affect growth rates, as neither initial densities ($F_{[1,28]} = 1.1$, $P = 0.30$) nor final densities ($F_{[1,28]} = 3.1$, $P = 0.09$) proved significant when included as covariates in the model.

Growth rate was measured as the standardized mass-specific growth rate Ω% (growth rate of a 1 g fish, Ostrovsky 1995):

$$(1) \qquad \Omega = \frac{M_t^b - M_0^b}{b \times t} \times 100$$

where M_0 and M_t is the tank-specific mean body mass (g wet mass) at the beginning and end of the experiment, respectively, t is the experimental period (in days), and b is the allometric mass exponent for the relationship between growth rate and body size (estimated to be 0.31 for Atlantic salmon juveniles, Elliott and Hurley 1997).

To assess the effects of temperature on the activity patterns of first feeding fry, time budget analysis of behaviour were performed on 10 fry in each temperature treatment by video recordings in separate aquaria. The video trials were performed on fish that had been kept in separate nests at the experimental temperatures parallel to the emergence experiment described above. The fry were transferred to 10 L tanks (one per temperature treatment) and fed with commercial fish pellets for a minimum 5 days prior to the video trial. The trials were performed during the period of peak emergence from the nests at each of the experimental temperatures, and only fry that were seen swimming active and appeared to have initiated feeding were chosen for the trials. Prior to the observations, the fry were starved for 24 h. One fry was transferred to each of ten 1 L aquaria for habituation for a minimum of 6 h before the trial. The aquaria used for the trial were provided with gravel on the bottom and had a water level of 8 cm. Black plastic film was attached to the sides of the aquarium to isolate the fry from visual disturbance. The video recordings were initiated by introducing ~30 food pellets onto the surface of the aquaria. The behaviour and activity of the fry were then monitored for 15 min, and the time used on each activity was later measured from the video recordings. The behaviours were first categorized as being either "active" or "inactive" (when the fry rest on the bottom of the aquaria continuously for more than 3 s). The time spent in activity was further divided into "active near bottom", when the fry move around in the lower half of the water column (within 4 cm from the bottom), and "active near surface", when the fry resided in the more exposed upper half of the water column. In addition, the total number of attacks on food particles on the surface was recorded.

Body composition

Fry sampled at emergence and from the growth experiment were defrosted, photographed, and weighed (wet mass: ±0.01 mg). Dry mass and water content was recorded after drying at 70 °C for 24 h. Fry length and the size of the yolk sac (surface area below the belly as seen from the side) were measured from the photographs (ImageJ, v.1.37, Rasband 1997–2005).

An additional 10 emerging fry from each nest were used for estimation of proximate composition (protein, fat, and ash content). The fry were selected by sampling fish regularly with respect to emergence date to obtain a representative sample from each nest. Each fry was then desiccated for 48 h at 55 °C. Fat content (lipids) was measured by mass loss after extraction in a petrol–ether solvent (5:1 *v/v*). Protein content was then determined as the difference between the mass of the fat-free body mass and the ashes after combustion (12 h, 550 °C), as described in Einum and Fleming (2000). Energy content of the fry was estimated by assigning caloric values of 39 and 24 $kJ \cdot g^{-1}$ for fat and protein, respectively (Jobling 1995). Forty-nine of the fry samples were lost during processing and hence not included in the analysis.

Statistical analysis

The statistical analyses were performed using R (v. 2.8.1, R Development Core Team 2008). Analyses for effects of temperature on survival in nests and tanks, and of activity time budgets, were performed by fitting generalized linear models (GLM) with binomial distributions. The number of food particles attacked in the video trials was analysed using a GLM with Poisson distribution. Owing to overdispersion, all GLM were refitted using quasi-GLM error structures and using *F* tests for significance testing (Crawley 2007).

Analysis of body size and composition was performed using linear mixed effects models with the lme4 package in R (Bates et al. 2008). For each of the response variables, a full model was first fitted with temperature treatments, relative date of emergence (standardized for each nest) and their interactions as fixed effects, and nests as a random effect. Residuals were graphically surveyed for normality, homoscedasticity, and outliers, and were found to satisfy the assumptions for the models. Values of yolk surface area were square root transformed, whereas water content and all mass-specific values of proximate composition were arcsine square root transformed. As calculating *P* values based on *F* ratios are nontrivial for these models (Bates 2006), a model selection procedure was applied with stepwise elimination of terms (in the following order: interaction, emergence date, temperature) from models fitted with maximum likelihood. Inferences of the fixed effects were then performed by comparison of models containing a fixed effect with a simpler model without the term, as judged by changes in Akaike information criterion (AIC) and likelihood ratio tests. For convenience, ΔAIC values are reported, where the model with lowest AIC value is set to 0. In addition, *P* values for the fixed effects were obtained by running a Markov chain Monte Carlo (MCMC) procedure with 10 000 simulations of models fitted with restricted maximum likelihood, and by resampling the posteriori distribution using the "languageR" package in R (Baayen 2008).

Results

Emergence from nests

Overall survival from the start of the experiment to emergence from the nests was 97.2%. While there appeared to be a minor effect of temperature on survival (GLM: $F_{[3,28]} = 2.7$, $P = 0.059$), this was most likely due to one nest in the 2 °C treatment with deviating low survival (82%). After excluding this nest, no trend was found in survival among temperature treatments (GLM: $F_{[3,27]} = 1.4$, $P = 0.27$).

Temperature treatments in the nests had a strong effect on emergence date, with emergence occurring progressively later and over a longer time period with decreasing temperature (Fig. 1). The mean observed median dates of emergence from the nests matched the predicted date based on an empirical development model (Crisp 1981, 1988) for the 5, 8, and 12 °C temperature treatments, but was on average 8 days later than predicted in the 2 °C treatment. However, all median emergence times were within the 95% confidence interval of the model, and timing of emergence was generally as predicted.

Compared to alevins at the start of the experiment, fry in all temperature groups had increased in length and water content, but decreased in wet mass, dry mass, yolk sac size, and total energy content when they emerged the nests (data not shown).

Comparison of the linear mixed models revealed that temperature had strong effects on body size and composition at emergence, as judged by the decrease in ΔAIC and significance levels when comparing the models with temperature against the null model in the model selection procedure (Table 2). Temperature had significant effects on body length ($P < 0.001$), wet mass ($P < 0.001$), dry mass ($P = 0.002$), water content ($P = 0.012$), and yolk sac size ($P < 0.001$) at emergence (*P* values from MCMC). In general, fry emerging from the low temperature treatments were smaller, had lower water content, and had larger yolk sacs than fry emerging from the higher temperature treatments (Fig. 2).

The effect of incubation temperature was also reflected in the composition of emerging fry (Table 3). Mass specific lipid contents were largest in the 2 °C treatment group (being the smallest in length and mass, but with the largest yolk sacs), and lowest in the 12 °C treatment group (Table 4). Whereas total energy content of fry was lowest in the 2 °C treatment and highest in the 8 °C treatment, the mass specific energy content (per wet mass) decreased significantly with increasing temperature (Table 4).

The body composition of fry also changed throughout the emergence period (Tables 2 and 3). Whereas dry body mass was similar across emergence dates, body length increased significantly through the period ($P < 0.001$). Significant interaction effects were found between temperature and emergence date for both wet mass ($P < 0.001$), water content ($P < 0.001$), and yolk sac size ($P = 0.001$). Both water content and wet mass showed positive slopes for emergence date in the 12 and 8 °C treatments, but no effects were seen in 2 and 5 °C treatments. Yolk sac size on the other hand, showed positive slopes for emergence date in the 5 and 8 °C treatments and negative slopes in the 2 and 12 °C treatments. Similarly, both wet mass specific fat levels ($P = 0.01$) and wet mass specific energy levels ($P = 0.027$) decreased significantly throughout the emergence period, but treatment contrasts showed that the effect was only significant in the 8 °C group.

Performance after emergence

Overall survival through the start feeding experiment (after excluding tanks with high mortality due to parasite infection, see Materials and methods) was 73.5%, and there was no difference in survival among temperature treatments (GLM: $F_{[3,23]} = 1.6$, $P = 0.22$).

Growth was positive and increased significantly with temperature (Fig. 3). Positive growth was also found at all temperatures in terms of dry mass and for body length in the 5, 8, and 12 °C treatments (paired sample *t* tests for means between nests and tanks: all $P < 0.01$), whereas no growth was found in body length at 2 °C (paired sample *t* tests: $t_7 = -0.15$, $P = 0.44$).

Temperature had a strong influence on the activity pattern of the first feeding fry. They spent considerable more time remaining inactive on the bottom of the test aquaria in the low temperatures compared to at the higher temperature treatments (GLM: $F_{[3,36]} = 20.7$, $P < 0.001$, Table 5). Further, during activity, a significantly higher proportion of time was spent in the upper half of the water column (near the surface) with increasing temperature (GLM: $F_{[3,36]} = 18.4$, $P < 0.001$). The number of attacks on food particles also in-

Fig. 1. The timing of emergence of Atlantic salmon fry from artificial nests in four temperature treatments. The bars represent median date (line), upper and lower quartiles (box), range (whiskers), and outliers (dots) from each of the artificial nests. The arrows indicate the date of median emergence predicted for each of the temperature treatments, based on an existing model for the relationship between temperature and development rate (Crisp 1981, 1988).

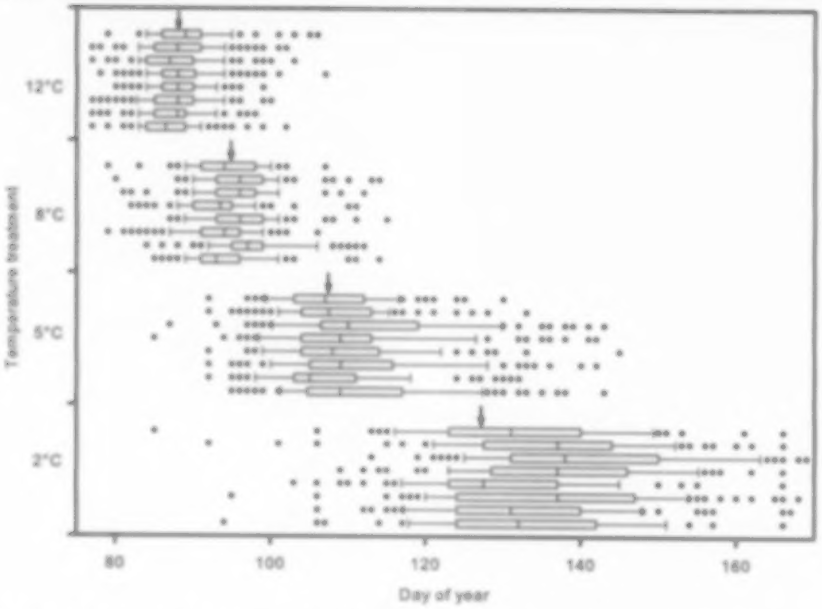


Table 2. Model summary of linear mixed models for responses on body composition parameters of emerging Atlantic salmon fry.

	ΔAIC of models with respect to different response parameters				
Model	Length	Wet mass	Dry mass	% water	Yolk area
T + E + T × E	0	0***	1.9	0***	0**
T + E	0.7***	16.4***	1.8	12.9***	10.2
T	13.5***	21.5***	0***	39.3***	8.4***
Null model (random intercept)	40.4	45.9	10.3	45.5	49.1

Note: All models include nest as a random effect. The uppermost (full) model includes temperature treatment (T), standardized emergence date (E), and their interaction term as fixed factors. The summary gives ΔAIC values for models as factors are sequentially deleted from the full model. Asterisks indicate significance levels for likelihood ratio tests when each model is compared against the simpler model on the line below: *, statistical significance at $p < 0.05$; **, statistical significance at $p < 0.01$; ***, statistical significance at $p < 0.001$.

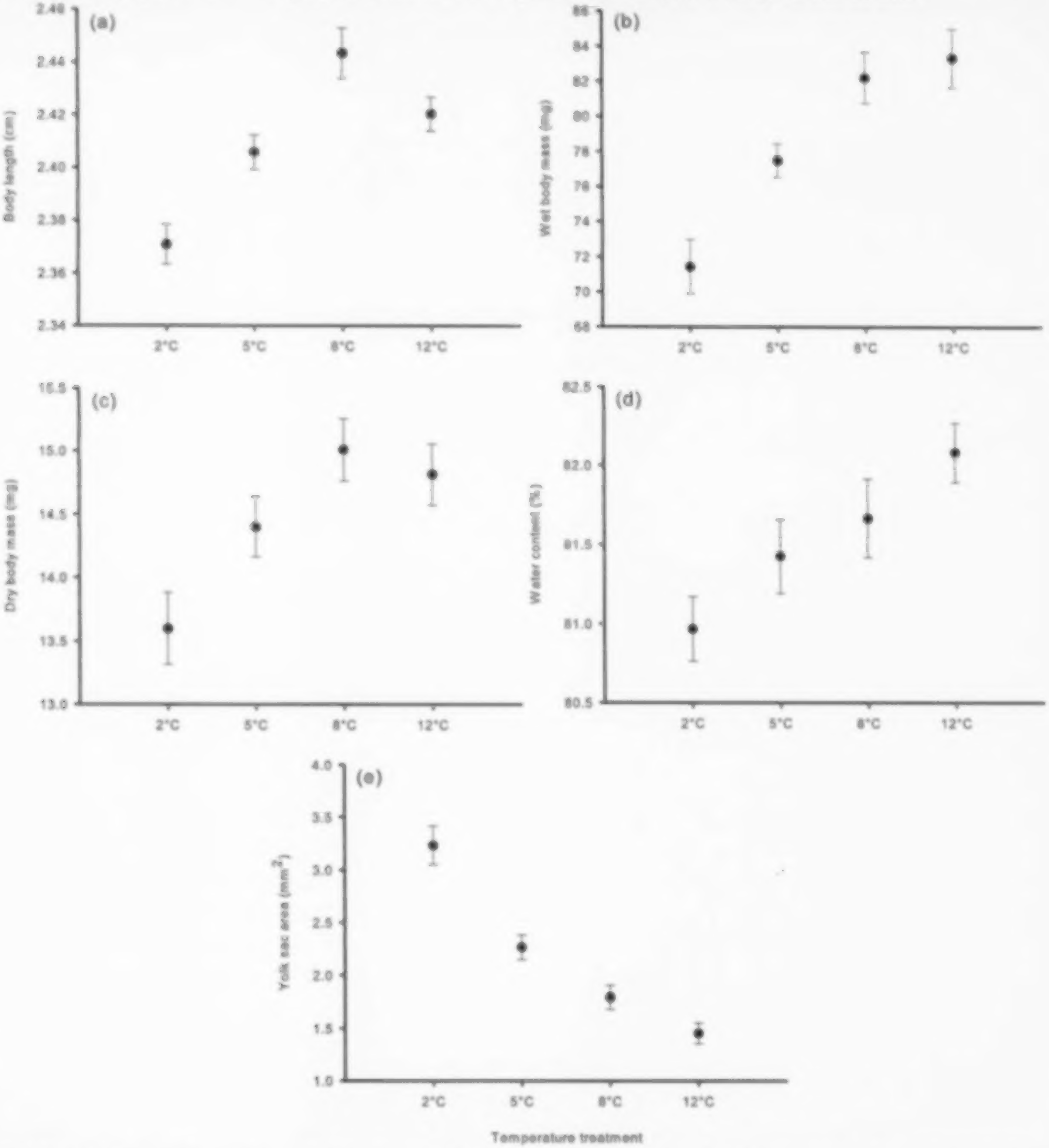
creased significantly with temperature (GLM: $F_{[3,36]} = 15.7$, $P < 0.001$, Table 5).

Discussion

Temperature is one of the most important environmental factors affecting performance of fish across life stages. In the present study, we tested how temperature affects phenotypes of Atlantic salmon juveniles emerging from nests, as well as postemergent activity, feeding, and growth during the critical period of initiation of exogenous feeding. Alevins experiencing lower temperatures during the terminal period of nest incubation were smaller but had larger yolk sacs and higher mass specific energy contents than alevins experiencing higher temperatures. Thus, temperature experienced in the days and weeks prior to emergence not only affected the development rate, and thus the timing of emergence, but also the size and physiological condition at the time when emergence was triggered.

Prior to initiating exogenous feeding, yolk constitutes the sole energy source and available yolk reserves are mainly partitioned between formation of tissue and respiration (Kamler 2008). Thus, whereas the size of the embryo increases as it grows during development, the total energy content of the alevins (embryo + yolk) decreases as some of the yolk is metabolized during incubation. The smaller dry mass and lower total energy content of the alevins incubated at low temperatures in the present study therefore reflects a higher metabolic expenditure prior to emergence. This may at first seem contradictory, as lower temperatures results in lowered metabolism and hence reduced metabolic requirements. However, lower temperatures also result in a prolonged development time, and the effect of temperature on the metabolic efficiency of turning yolk into tissue differs among fish species (reviewed by Kamler 2008). The emerging fry in the present study had the highest dry mass and total energy contents at 8 °C, suggesting that this is the most metabolically

Fig. 2. Mean ±1 SE (n = 8 nests per temperature) of body length (a), wet body mass (b), dry body mass (c), water content (d), and yolk sac area (e) of emerging Atlantic salmon fry exposed to four different temperature treatments prior to emergence from the nests.



efficient temperature for turning yolk into tissue. This also corresponds well with other studies that generally find that the largest total size of fry (after yolk exhaustion) are reached at post-hatching temperatures of about 10 °C for this species (Marr 1966; Hamor and Garside 1977; Peterson and Martin-Robichaud 1995). Despite the reduced metabolic efficiency of alevins incubated at low temperatures, these had more yolk remaining and higher mass specific energy contents at emergence. A larger yolk sac (and a smaller body size) indicates that the fry emerged at an earlier developmental stage, which also corresponds with the fry having lower water contents and in general appearing more precocious compared to fry emerging at the higher temperatures. With the present data, we were not able to reveal whether the mechanisms be-

Table 3. Model summary of linear mixed models for responses on proximate composition (percentage of dry mass) and energy content of emerging Atlantic salmon fry.

	ΔAIC of models with respect to different response parameters					
Model	Protein (%)	Lipids (%)	Ash (%)	Energy content (kJ)	Energy-dry mass^{-1} (kJ·g^{-1})	Energy-wet mass^{-1} (kJ·g^{-1})
T + E + T × E	1.6	0	1.6	3.6	0	5.2
T + E	1.9	1.6*	0**	0	0.0**	0*
T	0*	5.4*	5.5	0.6*	5.2	3.0***
Null model (random intercept)	2.0	9.8	2.4	4.2	6.3	24.7

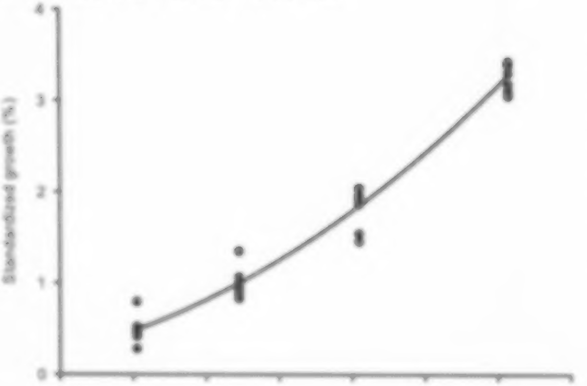
Note: All models include nest as a random effect. The uppermost (full) model includes temperature treatment (T), standardized emergence date (E), and their interaction term as fixed factors. The summary gives ΔAIC values for models as factors are sequentially deleted from the full model. Asterisks indicate significance levels for likelihood ratio tests when each model is compared against the simpler model on the line below: *, statistical significance at $p < 0.05$; **, statistical significance at $p < 0.01$; ***, statistical significance at $p < 0.001$.

Table 4. Proximate composition (as percentage of dry mass) and energy content of Atlantic salmon fry emerging from nests in four temperature treatments, and *P* values from Markov chain Monte Carlo (MCMC) for effect of temperature treatment from linear mixed models.

	Temperature treatments				
	2 °C (n = 59)	5 °C (n = 79)	8 °C (n = 77)	12 °C (n = 63)	P_{MCMC}
Proximate composition					
Protein (%)	77.5±4.1	78.5±3.6	78.6±2.9	80.4±2.6	0.019
Lipids (%)	14.3±4.0	12.8±3.2	13.6±3.2	11.8±3.2	0.007
Ash (%)	8.2±2.6	8.7±2.5	7.8±2.0	7.8±1.8	0.242
Energy content					
Energy content (kJ)	0.34±0.08	0.36±0.08	0.39±0.08	0.36±0.08	0.021
Wet-mass specific energy (kJ ·g^{-1})	5.12±0.81	4.90±0.81	4.57±0.60	4.35±0.57	<0.001

Note: Values are given as arithmetic means ± SD pooled within temperature treatments.

Fig. 3. Relationship between mass-specific standardized growth rate (Ω) and temperature for first feeding Atlantic salmon fry. Each point represents average growth from one tank. A polynomial model provided a good fit to the relationship ($0.0124x^2 + 0.0945x + 0.237$, $R^2 = 0.98$, $F_{[2,29]} = 635.1$, $P < 0.0001$).



hind this involved adjusting the ontogenetic timing of emergence, or were a result of developmental allocation processes (i.e., being at the same developmental stage but having invested less of the yolk into tissue synthesis and growth). Nevertheless, the results clearly demonstrate phenotypic plasticity in the amount of yolk remaining, and hence the energetic status at emergence in response to temperature experienced in the terminal period of nest incubation.

The observed differences in body size and amount of yolk at emergence are likely to have fitness consequences. Body size is considered one of the most important factors affecting survival during larval and early juvenile stages (Miller et al. 1988), as large individuals generally are less susceptible to size-selective predation (Anderson 1988) and have shorter durations of vulnerable stages (Hare and Cowen 1997). In addition, body size is often positively related to competitive abilities (Cutts et al. 1999) and hence survival in territorial species such as Atlantic salmon (Einum and Fleming 2000). This suggests that individuals should maximize body size given the available amount of yolk reserves. On the other hand, depleted endogenous energy reserves may increase the risk of starvation (Chambers et al. 1989; Berkeley et al. 2004; Fisher et al. 2007), and emerging with yolk reserves remaining is likely to provide an energetic buffer during the critical transition to exogenous feeding. Hence, the body size and physiological condition at emergence could be viewed as an allocation trade-off between having large energy stores (and hence starvation resistance) versus large body size (better locomotory ability and predation avoidance). Letcher and Terrick (2001) found that unfed Atlantic salmon fry stocked in the rivers at a developmentally accelerated stage showed reduced survival, most likely owing to a high probability of starvation. Further, Bailey et al. (2010) found a strong stabilizing selection when manipulating the development stage at release of Atlantic salmon alevins and fry in streams, showing negative fitness consequences of both precocious and advanced development stages when emergence is "forced" by direct release into the river.

Can the phenotypic plasticity observed in the present study

Table 5. Average time in percent (with range) Atlantic salmon fry spent as inactive, and active in lower (bottom) and upper (surface) part of the test aquaria, proportion of fry observed feeding, and average (range) number of food pellets taken during behavioural observations within each temperature treatment.

		Active			
Temperature (°C)	Inactive (%)	Bottom (%)	Surface (%)	Proportion of fry feeding	No. food pellets
2	91.6 (74.3–100)	6.5 (0.0–21.2)	1.9 (0.0–4.7)	0	0.0 (0–0)
5	46.8 (4.0–99.1)	42.5 (0.9–71.3)	10.8 (0.0–27.6)	0	0.0 (0–0)
8	6.9 (0.0–45.1)	69.6 (43.0–83.7)	23.5 (11.9–43.6)	0.9	2.2 (0–6)
12	19.0 (0.0–51.1)	45.0 (33.2–61.7)	36.0 (15.7–59.9)	0.7	5.8 (0–22)

be adaptive? Emergence from nests is not a development event in itself, but can be viewed as a behaviourally based habitat shift. It therefore seems unlikely that fry should be physiologically constrained to emerge with a larger yolk sac when temperatures are low. At low temperatures, the newly emerged fry were found to remain close to or hide within the bottom substratum. Although this may be a consequence of reduced metabolism on activity at low temperatures, this behaviour is also likely to reduce predation risk of salmonid fry (Brännäs 1995). Similar reduced activity and increased sheltering behaviour occurs in older salmon juveniles when temperatures drop during the autumn and winter (e.g., Fraser et al. 1993). Furthermore, this behaviour depends on the individual energy status, with individuals with low energy levels increasing activity and feeding until a certain defended energy threshold is reached (Metcalfe and Thorpe 1992; Bull et al. 1996). A similar mechanism may represent an adaptive explanation for the larger yolk sac of fry emerging at low temperatures. By emerging with larger endogenous energy stores and a smaller metabolizing body mass, the fry have reduced the immediate need for exogenous food after emergence, may endure for a period with lower activity levels, and perhaps feed more cryptically on benthic prey, when temperatures are low. Cryptic feeding and sheltering behaviour in the substrate under low temperatures may also reduce the importance of body size, which is primarily of importance in competitive interactions (Einum and Fleming 1999). A similar pattern of allocation is seen among species of rockfish larvae (*Sebastes* spp.), where species that spawn during winter have smaller larval size but larger endogenous lipid reserves than those spawning during the more productive spring season (Fisher et al. 2007). Furthermore, whereas the former show a higher starvation resistance, the latter have better swimming performance. Yet, it remains to be tested whether the optimal developmental stage, in terms of residual yolk at emergence, depends on temperature in Atlantic salmon.

It was also found that low temperatures resulted in the emergence period being extended and consequently less synchronous at low temperatures. Reduced synchrony may affect offspring performance in several ways. First, a more protracted period of emergence may buffer against the effects of single events that may cause mortality of newly emerged offspring, such as episodes of high discharge (Jensen and Johnsen 1999). Second, a larger asymmetry in competition may develop as the earliest juveniles may gain an increasing competitive advantage in terms of body size and prior residence effects (Kvingedal and Einum 2011), and also cause increased life history variation among individuals in terms of smolt age and parr maturation (Metcalfe 1998). Finally, less synchronous emergence timing may cause a higher predation risk if predation is imposed by small predators that rapidly achieve satiation (Ward et al. 2008).

In seasonal environments, the environmental conditions experienced during critical periods of the life cycle will, to a large degree, depend on the phenology. There is increasing concern that changes in seasonal parameters (e.g., due to climate change or other human perturbation) may cause a shift in the phenology and cause a mismatch between phenology and optimal conditions for offspring (Visser et al. 1998; Thackeray et al. 2010). In the case of Atlantic salmon, the timing of emergence from nests will depend largely upon the timing of spawning and the temperature conditions experienced during incubation. If, for example, winter temperatures increase, which is a likely scenario both under global warming and for many types of hydropower regulations, this may cause emergence to occur relatively earlier in the season, and may (depending on the acceleration of development compared to the spring increase in temperature) result in temperatures becoming lower during initiation of feeding. In the present study, we found that temperature had strong effects on activity, feeding, and growth, but low temperatures did not represent any physiological limitation for initiation of feeding. Further, lower temperatures experienced prior to emergence from the nests caused fry to emerge at a smaller size but with a larger yolk sac, and hence with more endogenous energy reserves. Whereas the exact response may differ somewhat in natural situations where both temperature and photoperiod changes during the season, and although the adaptive significance of this response still is to be documented, we propose that this behaviour may be an important mechanism to deal with interannual environmental variation. The allocation trade-off between body size and energy stores in juvenile life stages has recently gained increasing interest in a life history perspective, and several studies have found organisms capable to respond to this trade-off in a facultative and state-dependent manner (Post and Parkinson 2001; Metcalfe et al. 2002; Hurst and Conover 2003). The present study adds to this field by highlighting the possibility for phenotypic plasticity in energy allocation even prior to the onset of exogenous feeding.

Acknowledgements

The authors thank the staff at NINA Research Station Ims, and especially Tormod Husebø for assistance during the experiment, Anne Lohrmann for analyses of proximate composition, and Per J. Jakobsen for commenting on an earlier draft of the manuscript. Financial support was provided from the Norwegian Research Council through the Clean energy for the future (RENERGI) program (project No. 177893); and the Norwegian Directorate for Nature Management, Statkraft Energi AS, Agder Energy Production, and BKK and the Norwegian Electricity Industry Association. The study was conducted according to national regulations for the treatment and welfare of experimental animals.

References

Anderson, J.T. 1988. A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. J. Northwest Atl. Fish. Sci. **8**: 55–66.

Baayen, R.H. 2008. languageR: data sets and functions with "analyzing linguistic data: a practical introduction to statistics" [online]. R package version 0.953. Available from http://cran.r-project.org/web/packages/languageR/ [accessed 15 June 2009].

Bailey, M.M., Lachapelle, K.A., and Kinnison, M.T. 2010. Ontogenetic selection on hatchery salmon in the wild: natural selection on artificial phenotypes. Evol. Appl. **3**(4): 340–351. doi:10.1111/j.1752-4571.2009.00115.x.

Bates, D. 2006. lmer, p-values and all that [online]. Available from finzi.psych.upenn.edu/R/Rhelp02a/archive/76742.html [accessed 15 June 2009].

Bates, D., Maechler, M., and Dai, B. 2008. lme4: linear mixed-effects models using S4 classes [online]. R package version 0.999375–28. Available from http://cran.r-project.org/web/packages/lme4/ [accessed 15 June 2009].

Berkeley, S.A., Chapman, C., and Sogard, S.M. 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology, **85**(5): 1258–1264. doi:10.1890/03-0706.

Brännäs, E. 1995. First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. Evol. Ecol. **9**(4): 411–420. doi:10.1007/BF01237763.

Bull, C.D., Metcalfe, N.B., and Mangel, M. 1996. Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. Proc. R. Soc. B Biol. Sci. **263**(1366): 13–18.

Chambers, R.C., Leggett, W.C., and Brown, J.A. 1989. Egg size, female effects, and the correlations between early life-history traits of capelin, *Mallotus villosus*: an appraisal at the individual level. Fish Bull. **87**(3): 515–523.

Crawley, M.J. 2007. The R book. John Wiley & Sons Ltd., Chichester, UK.

Crecco, V.A., and Savoy, T.F. 1985. Effects of biotic and abiotic factors on growth and relative survival of young American shad, *Alosa sapidissima*, in the Connecticut River. Can. J. Fish. Aquat. Sci. **42**(10): 1640–1648. doi:10.1139/f85-205.

Crisp, D.T. 1981. A desk study of the relationship between temperature and hatching time for the eggs of five species of salmonid fishes. Freshw. Biol. **11**(4): 361–368. doi:10.1111/j.1365-2427.1981.tb01267.x.

Crisp, D.T. 1988. Prediction, from temperature, of eying, hatching and 'swim-up' times for salmonid embryos. Freshw. Biol. **19**(1): 41–48. doi:10.1111/j.1365-2427.1988.tb00325.x.

Cushing, D.H. 1990. Plankton production and year-class strength in fish populations: an update of the match mismatch hypothesis. Adv. Mar. Biol. **26**: 249–293. doi:10.1016/S0065-2881(08)60202-3.

Cutts, C.J., Metcalfe, N.B., and Taylor, A.C. 1999. Competitive asymetries in territorial juvenile Atlantic salmon, *Salmo salar*. Oikos, **86**(3): 479–486. doi:10.2307/3546652.

Einum, S., and Fleming, I.A. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. Proc. R. Soc. B Biol. Sci. **266**(1433): 2095–2100.

Einum, S., and Fleming, I.A. 2000. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). Evolution, **54**(2): 628–639. PMID:10937238.

Elliott, J.M., and Hurley, M.A. 1997. A functional model for maximum growth of Atlantic salmon parr, *Salmo salar*, from two populations in northwest England. Funct. Ecol. **11**(5): 592–603. doi:10.1046/j.1365-2435.1997.00130.x.

Finstad, A.G., Næsje, T.F., and Forseth, T. 2004. Seasonal variation in the thermal performance of juvenile Atlantic salmon (*Salmo salar*). Freshw. Biol. **49**(11): 1459–1467. doi:10.1111/j.1365-2427.2004.01279.x.

Fisher, R., Sogard, S.M., and Berkeley, S.A. 2007. Trade-offs between size and energy reserves reflect alternative strategies for optimizing larval survival potential in rockfish. Mar. Ecol. Prog. Ser. **344**: 257–270. doi:10.3354/meps06927.

Forseth, T., Hurley, M.A., Jensen, A.J., and Elliott, J.M. 2001. Functional models for growth and food consumption of Atlantic salmon parr, *Salmo salar*, from a Norwegian river. Freshw. Biol. **46**(2): 173–186. doi:10.1046/j.1365-2427.2001.00631.x.

Fraser, N.H.C., Metcalfe, N.B., and Thorpe, J.E. 1993. Temperature-dependent switch between diurnal and nocturnal foraging in salmon. Proc. R. Soc. B Biol. Sci. **252**(1334): 135–139.

García De Leániz, C., Fraser, N., and Huntingford, F.A. 2000. Variability in performance in wild Atlantic salmon, *Salmo salar* L., fry from a single redd. Fish. Manag. Ecol. **7**(6): 489–502. doi:10.1046/j.1365-2400.2000.00223.x.

Hamor, T., and Garside, E.T. 1977. Size relations and yolk utilization in embryonated ova and alevins of Atlantic salmon *Salmo salar* L. in various combinations of temperature and dissolved oxygen. Can. J. Zool. **55**(11): 1892–1898. doi:10.1139/z77-242. PMID:563282.

Hare, J.A., and Cowen, R.K. 1997. Size, growth, development, and survival of the planktonic larvae of *Pomatomus saltatrix* (Pisces: Pomatomidae). Ecology, **78**(8): 2415–2431.

Heming, T.A. 1982. Effects of temperature on utilization of yolk by chinook salmon (*Oncorhynchus tshawytscha*) eggs and alevins. Can. J. Fish. Aquat. Sci. **39**(1): 184–190. doi:10.1139/f82-021.

Hurst, T.P., and Conover, D.O. 2003. Seasonal and interannual variation in the allometry of energy allocation in juvenile striped bass. Ecology, **84**(12): 3360–3369. doi:10.1890/02-0562.

Jensen, A.J., and Johnsen, B.O. 1999. The functional relationship between peak spring floods and survival and growth of juvenile Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). Funct. Ecol. **13**(6): 778–785. doi:10.1046/j.1365-2435.1999.00358.x.

Jobling, M. 1995. Environmental biology of fishes. Chapman and Hall, London, UK.

Jones, M., Laurila, A., Peuhkuri, N., Piironen, J., and Seppa, T. 2003. Timing an ontogenetic niche shift: responses of emerging salmon alevins to chemical cues from predators and competitors. Oikos, **102**(1): 155–163. doi:10.1034/j.1600-0706.2003.12347.x.

Jonsson, B., Forseth, T., Jensen, A.J., and Næsje, T.F. 2001. Thermal performance of juvenile Atlantic salmon, *Salmo salar* L. Funct. Ecol. **15**(6): 701–711. doi:10.1046/j.0269-8463.2001.00572.x.

Kamler, E. 1992. Early life history of fish: an energetics approach. Chapman and Hall, London, UK.

Kamler, E. 2008. Resource allocation in yolk-feeding fish. Rev. Fish Biol. Fish. **18**(2): 143–200. doi:10.1007/s11160-007-9070-x.

Kvingedal, E., and Einum, S. 2011. Prior residency advantage for Atlantic salmon in the wild: effects of habitat quality. Behav. Ecol. Sociobiol. . doi:10.1007/s00265-011-1143-0.

Letcher, B.H., and Terrick, T.D. 2001. Effects of developmental stage at stocking on growth and survival of Atlantic salmon fry. N. Am. J. Fish. Manage. **21**(1): 102–110. doi:10.1577/1548-8675(2001)021<0102:EODSAS>2.0.CO;2.

Marr, D.H.A. 1966. Influence of temperature on the efficiency of growth of salmonid embryos. Nature, **212**(5065): 957–959. doi:10.1038/212957a0. PMID:6012332.

Metcalfe, N.B. 1998. The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. **55**(S1 Suppl. 1): 93–103. doi:10.1139/cjfas-55-S1-93.

Metcalfe, N.B., and Thorpe, J.E. 1992. Anorexia and defended energy-levels in over-wintering juvenile salmon. J. Anim. Ecol. **61**(1): 175–181. doi:10.2307/5520.

Metcalfe, N.B., Bull, C.D., and Mangel, M. 2002. Seasonal variation in catch-up growth reveals state-dependent somatic allocations in salmon. Evol. Ecol. Res. **4**(6): 871–881.

Miller, T.J., Crowder, L.B., Rice, J.A., and Marschall, E.A. 1988. Larval size and recruitment mechanisms in fishes: towards a conceptual framework. Can. J. Fish. Aquat. Sci. **45**(9): 1657–1670. doi:10.1139/f88-197.

Nislow, K.H., and King, D.I. 2006. Transition from maternal provisioning: crunch time in vertebrate life histories? J. Zool. **269**(4): 401–402. doi:10.1111/j.1469-7998.2006.00162.x.

Ojanguren, A.F., Reyes-Gavilan, F.G., and Munoz, R.R. 1999. Effects of temperature on growth efficiency of yolk utilisation in eggs and pre-feeding larval stages of Atlantic salmon. Aquacult. Int. **7**(2): 81–87. doi:10.1023/A:1009214804949.

Ostrovsky, I. 1995. The parabolic pattern of animal growth — determination of equation parameters and their temperature dependencies. Freshw. Biol. **33**(3): 357–371. doi:10.1111/j.1365-2427.1995.tb00398.x.

Peterson, R.H., and Martin-Robichaud, D.J. 1989. First feeding of Atlantic salmon (*Salmo salar* L.) fry as influenced by temperature regime. Aquaculture, **78**(1): 35–53. doi:10.1016/0044-8486(89)90004-5.

Peterson, R.H., and Martin-Robichaud, D.J. 1995. Yolk utilization by Atlantic salmon (*Salmo Salar* L.) alevins in response to temperature and substrate. Aquacult. Eng. **14**(1): 85–99. doi:10.1016/0144-8609(94)P4428-E.

Post, J.R., and Parkinson, E.A. 2001. Energy allocation strategy in young fish: allometry and survival. Ecology, **82**(4): 1040–1051. doi:10.1890/0012-9658(2001)082[1040:EASIYF]2.0.CO;2.

R Development Core Team. 2008. R: a language and environment for statistical computing [online]. R Foundation for Statistical Computing, Vienna, Austria. Available from www.R-project.org [accessed 8 May 2009].

Rasband, W. 1997–2005. ImageJ. US National Institutes of Health, Bethesda, Maryland, USA. Available from http://rsb.info.nih.gov/ij/ [accessed 12 March 2007].

Skoglund, H., and Barlaup, B.T. 2006. Feeding pattern and diet of first feeding brown trout fry under natural conditions. J. Fish Biol. **68**(2): 507–521. doi:10.1111/j.0022-1112.2006.00938.x.

Thackeray, S.J., Sparks, T.H., Frederiksen, M., Burthe, S., Bacon, P.J., Bell, J.R., Botham, M.S., Brereton, T.M., Bright, P.W., Carvalho, L., Clutton-Brock, T., Dawson, A., Edwards, M., Elliott, J.M., Harrington, R., Johns, D., Jones, I.D., Jones, J.T., Leech, D.I., Roy, D.B., Scott, W.A., Smith, M., Smithers, R.J., Winfield, I.J., and Wanless, S. 2010. Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. Glob. Change Biol. **16**(12): 3304–3313. doi:10.1111/j.1365-2486.2010.02165.x.

Visser, M.E., van Noordwijk, A.J., Tinbergen, J.M., and Lessells, C.M. 1998. Warmer springs lead to mistimed reproduction in great tits (*Parus major*). Proc. R. Soc. B Biol. Sci. **265**(1408): 1867–1870.

Vonesh, J.R., and Warkentin, K.M. 2006. Opposite shifts in size at metamorphosis in response to larval and metamorph predators. Ecology, **87**(3): 556–562. doi:10.1890/05-0930. PMID:16602285.

Ward, D.M., Nislow, K.H., and Folt, C.L. 2008. Predators reverse the direction of density dependence for juvenile salmon mortality. Oecologia, **156**(3): 515–522. doi:10.1007/s00442-008-1011-4. PMID:18317816.

Wright, P.J., and Trippel, E.A. 2009. Fishery-induced demographic changes in the timing of spawning: consequences for reproductive success. Fish Fish. **10**(3): 283–304.

Biogeochemical responses of two alpine lakes to climate change and atmospheric deposition, Jasper and Banff National parks, Canadian Rocky Mountains

William O. Hobbs, Rolf D. Vinebrooke, and Alexander P. Wolfe

Abstract: The sensitivity of remote alpine ecosystems to global change has been documented by 20th century changes in climate, glacial recession, and terrestrial and aquatic ecosystems. Here we present sedimentary records of biogeochemical responses in two alpine lake ecosystems to multiple environmental drivers over the last ~500 years in Banff and Jasper National Parks (Alberta, Canada). We combine paleoecological measures of primary production and diatom community structure with geochemical proxies of reactive N (Nr) deposition to describe the nature and rate of recent ecosystem changes. Curator Lake in Jasper shows a strong diatom response to the limnological effects of climate warming (e.g., thermal stratification), but little evidence of changes in Nr cycling over the last ~500 years. The response of McConnell Lake in Banff to climate change is strongly mediated by glacial activity within the catchment and changing inputs of Nr. Our findings highlight the range of limnological responses that may be expressed by similar ecosystems subjected to comparable abiotic stressors, while further documenting the magnitude of the ecological footprint associated with recent environmental change in mountain park environments.

Résumé : La sensibilité des écosystèmes alpins éloignés au changement global a été démontrée par les modifications produites au cours du 20^e siècle dans le climat, dans le retrait des glaciers et dans les écosystèmes terrestres et aquatiques. Nous présentons ici des enregistrements sédimentaires de réponses biogéochimiques de deux écosystèmes lacustres alpins aux multiples facteurs de changement du milieu au cours des derniers ~500 ans dans les parcs nationaux de Banff et de Jasper (Alberta, Canada). Nous combinons des mesures paléoécologiques de la production primaire et des analyses de structure des communautés de diatomées à des variables géochimiques de remplacement du dépôt de N réactif (Nr) afin de décrire la nature et le taux des changements récents à l'écosystème. Le lac Curator à Jasper montre une forte réponse des diatomées aux effets limnologiques du réchauffement du climat (par ex., la stratification thermique), mais peu d'indications de changements dans le recyclage de Nr au cours des derniers ~500 ans. La réponse du lac McConnell à Banff au changement climatique est fortement influencée par l'activité glaciaire au sein du bassin versant et par les apports changeants de Nr. Nos résultats soulignent l'étendue des réponses limnologiques qui peuvent être exprimées par des écosystèmes semblables soumis à des facteurs abiotiques de stress comparables; ils apportent des informations additionnelles sur l'importance de l'empreinte écologique associée au changement récent dans les environnements montagneux des parcs.

[Traduit par la Rédaction]

Introduction

Alpine lakes are both sentinels and integrators of anthropogenic environmental change, despite their remote locations (Williamson et al. 2009). Profound ecological shifts in the ecosystems of alpine lakes have occurred during the 20th century (Parker et al. 2008; Hobbs et al. 2010*a*). Cumulative impacts of environmental changes on aquatic systems can be confounded by antagonistic and synergistic interactions among stressors (Vinebrooke et al. 2004). It therefore becomes necessary to expect synergistic responses of alpine lakes to regionally diffuse stressors such as climate change and the atmospheric deposition of nutrients. However, with a general lack of long term monitoring data on alpine lakes, it is often not possible to decipher how aquatic ecosystems have responded to stressors through time on a decadal to centennial scale. The careful interrogation of lake sediment records, paleolimnology, can be used to provide ecological trends and answers to questions of ecosystem response (Smol 2010). Geochemical and subfossil biological remains of the aquatic ecosystem are generally well preserved in lake sediments. Alpine lakes are well suited for paleolimnological

---

Received 21 September 2010. Accepted 25 March 2011. Published at www.nrcresearchpress.com/cjfas on 24 August 2011.
J22026

Paper handled by Associate Editor Gesa Antoine Weyhenmeyer.

W.O. Hobbs* and A.P. Wolfe. Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, AB T6G 2E3, Canada.
R.D. Vinebrooke. Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.

Corresponding author: William O. Hobbs (e-mail: whobbs@smm.org).

*Present address: St. Croix Watershed Research Station, 16910 152nd Street North, Marine-on-St. Croix, MN 55047, USA.

doi:10.1139/F2011-058 Published by NRC Research Press

studies of recent environmental change, as they are headwater ecosystems having small catchments relatively unaffected by local human perturbations. Therefore the signal to noise ratio of external anthropogenic stressors is considerably higher than lower elevation lakes. There are few paleolimnological investigations of lakes in the Canadian Rocky Mountains and they have focused mainly on long-term environmental change over millennial timescales, inferred from sedimentology and diatoms (Reasoner and Hickman 1989; Desloges and Gilbert 1995; Karst-Riddoch et al. 2005). Further background on paleolimnological approaches and the specific proxies used in this paper can be found in Last and Smol (2001) and Smol et al. (2001).

Climate history of the Canadian Rocky Mountains over the last 500 years has varied both spatially and temporally, inferred mainly through glacial geomorphology and dendroclimatology (Luckman 2000). The Little Ice Age (LIA), known as the Cavell Advance in the Canadian Rocky Mountains, represents a period of glacier fluctuations due to cold temperatures and increased precipitation, which began in the 13th century. The regional timing of the maximum glacial extent during the LIA varies from the early 18th century in the north (Jasper National Park, Alberta) to the mid-19th century in the south (Banff National Park, Alberta) (Luckman 2000). General culmination of the LIA occurred during the late 1800s and, while glacial response was asynchronous, the ablation and demise of many alpine glaciers in the Canadian Rocky Mountains has continued throughout the 20th and 21st centuries, with late 20th century global warming as a strong driver (Watson and Luckman 2004; Schindler and Donahue 2006).

Since the mid-1900s, the global flux of N to the atmosphere from industrial fixation has been more than the sum of all natural processes (Falkowski et al. 2000; Davidson 2009). In many alpine regions of North America and Europe, the deposition of anthropogenic reactive N (hereinafter Nr; comprising all biologically, photochemically, and radiatively active nitrogenous compounds in the atmosphere and biosphere; Galloway et al. 2004) has stimulated algal growth and significant compositional shifts in algal communities (Wolfe et al. 2003; Bergström and Jansson 2006; Elser et al. 2009*a*). The potential for atmospheric deposition of Nr has been considered low in the Canadian Rocky Mountains (Schindler et al. 2006), and more recently, survey- and bioassay-based tests confirm that N limitation is rare in lakes of this region (Murphy et al. 2010). Similarly, sediment diatom assemblages in alpine lakes immediately south of the Canada–USA border suggest that Nr deposition rates have not reached levels attained in the southern USA Rocky Mountains (Saros et al. 2011). However, terrestrial critical loads for Nr deposition have been exceeded in this region and atmospheric deposition of industrial pollutants have been documented in lake sediments (Landers et al. 2008).

In this study we combine paleoecological measures of algal production (sedimentary pigments) and community structure (fossil diatoms) with geochemical proxies of Nr deposition (stable N isotopes), sedimentology, and instrumental climate data to describe the response of two alpine lakes in the Canadian Rocky Mountains over the last ~500 years. We consider whether biotic changes are occurring in concert with climate change and ask whether climate is the sole driver of ecological change. We found that multiple abiotic drivers can be disentangled from the sediment record when taking into account the mediating influence of catchment processes, in particular glacial activity.

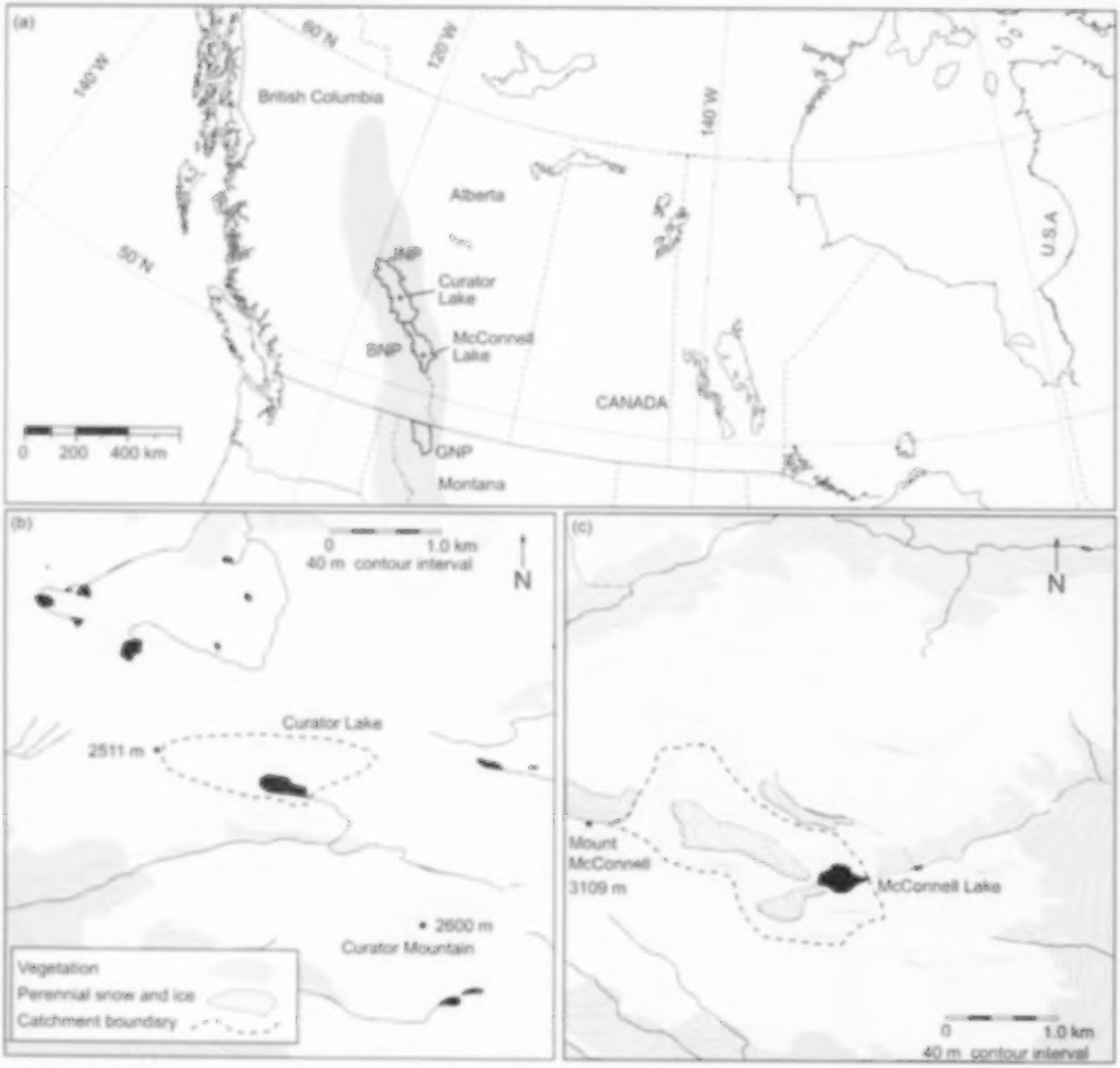
Materials and methods

Study sites

Curator Lake (52.79°N, 117.86°W) is situated in Jasper National Park, Alberta, Canada, south of Jasper townsite, within the Maligne Mountain Range (Fig. 1; Table 1). It is a deep, fishless, dilute, and alkaline alpine lake with an area of 0.05 km^2 (Table 1). There are no discernible inlets to the lake, suggesting that groundwater flow and interactions with talus are hydrologically important. One outlet, at the southern end of the lake, is a tributary to the Athabasca River. The small catchment has a morphology typical of alpine cirque basins (Fig. 2), but is unusual owing to its southern aspect (Fig. 1). There is currently no glacial ice visible within the catchment, however, two rock-glacierized debris flow lobes are visible on talus of the eastern and western slopes (Fig. 2). The Curator Lake catchment contains no obvious LIA terminal moraine. Centre Glacier, which lies approximately 4 km to the northwest, reached a LIA maximum at 1500 AD, and additional morainal deposits are present from 1650, 1808, 1862, and 1915 (Luckman 2000). Bedrock geology of the catchment includes Miette Group (Ediacaran) argillaceous sandstone and conglomerates, and minor dolomite and limestone. However, given the alkalinity and pH of the lake, it would seem that surrounding Lower Paleozoic bedrock, consisting mainly of thick-bedded limestone and dolomite, has a stronger influence on the chemistry of Curator Lake. The basin is primarily talus and bedrock, with a small area of vegetation adjacent to the eastern shore of the lake, which includes grasses, mosses, and lichens.

McConnell Lake (51.63°N, 115.98°W) is located in Banff National Park, Alberta, north–northwest of the Banff townsite, within the Vermillion Range (Fig. 1). Like Curator Lake, it is deep, fishless, dilute, and alkaline, with a similar lake area of 0.08 km^2 (Table 1). However, McConnell Lake has a much larger catchment than Curator Lake, with a northern aspect (Fig. 1). Bedrock geology of the catchment is Lower Paleozoic pale grey thick-bedded dolomite and limestone with calcareous and siliceous shales and local conglomerates. A cirque basin, occupied by a glacier during the LIA and currently contains a perennial snowpack, is perched to the south of McConnell Lake (Fig. 2). The catchment also extends to the west and contains perennial snowpack (Fig. 1). The complete LIA geomorphic activity has not been established for this basin; however, the nearby Drummond Glacier is thought to have reached a LIA maximum in the late 16th to mid-17th century (Luckman 2000). Changes in sedimentation to the nearby Hector Lake during glacial transitional periods suggest advances during the early 1700s and 1800s with rapid glacial recession in the early 20th century (Leonard 1997). A visible surface water input from the cirque basin enters the lake from the south, whereas the outlet to the lake is at the northern end (Fig. 2). The lake is a headwater system for McConnell Creek, which drains into the Red Deer River. The catchment is sparsely vegetated (<~10%) with shrubs, grasses, mosses, and a small copse of

Fig. 1. (*a*) Location of study sites. Approximate extent of the Rocky Mountain Range shaded in grey. Relevant national park boundaries for Jasper National Park (JNP), Canada; Banff National Park (BNP), Canada; and Glacier National Park (GNP), USA. (*b*) Topography of the Curator Lake basin, JNP with approximate area of the lake catchment shown with dashed line. (*c*) Catchment of McConnell Lake, BNP also showing lake catchment outline. Additional details for each lake are located in Table 1.


(a)
140°W
60°N
120°W
British Columbia
140°W
Alberta
50°N
JNP
Curator Lake
BNP
McConnell Lake
CANADA
U.S.A.
0 200 400 km
GNP
Montana
(b)
0 1.0 km
40 m contour interval
N
Curator Lake
2511 m
2600 m
Curator Mountain
Vegetation
Perennial snow and ice
Catchment boundary
(c)
N
Mount McConnell 3109 m
McConnell Lake
0 1.0 km
40 m contour interval

subalpine fir (*Abies lasiocarpa*) concentrated mainly on the western–northwestern side of the lake (Fig. 2).

Sediment core collection and chronology

A sediment core was retrieved from the deepest section of each lake (Table 1) using a Glew-type gravity corer (Glew et al. 2001). Sediment was subsampled immediately after recovery at 0.25 cm intervals for the first 10 and 0.5 cm intervals thereafter. Sediments were frozen within 24 h and freeze-dried soon after.

Geochronology was established through ^{210}Pb decay (measured as ^{210}Po) by alpha spectroscopy on each core (Appleby 2010). The constant rate of supply (CRS) model was used to estimate ages and sedimentation rate based on the radioactive decay of excess (or unsupported) ^{210}Pb above the background (or supported) ^{210}Pb activity (Appleby and Oldfield 1978).

Diatom microfossils

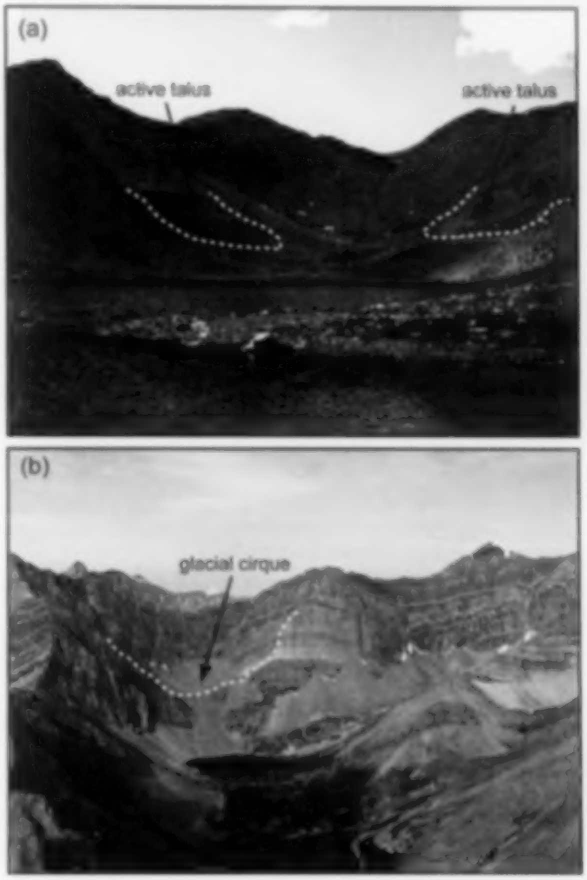
Sediments were processed using standard techniques for the oxidation of both carbonate minerals and organic matter, and permanently mounted on slides for diatom enumeration (Battarbee et al. 2001). Diatoms were enumerated under oil immersion (numerical aperture = 1.4) at a magnification of 1000× using differential interference contrast optics. Diluted

Table 1. Lake physical characteristics and water chemistry.

	Curator Lake	McConnell Lake
Latitude (°N)	52.792055	51.638162
Longitude (°W)	117.862727	115.977579
Altitude (m above sea level)	2232	2300
Lake area (km^2)	0.05	0.08
Catchment area (km^2)	0.8	2.7
Catchment : lake area	15.8	33.6
Maximum depth (m)	25	31
pH	8.4	8.0
Conductivity (µS·cm^{-1})	220	—
Turbidity (NTU)	0.69	—
DOC (mg·L^{-1})	—	0.6
NO_3^{-1}-N (µg·L^{-1})	66.5	129
Total N (µg·L^{-1})	269	184
Total P (µg·L^{-1})	3.98	3.00
DIN:TP	37	92
TN:TP	150	131

Note: Water chemistry represents a single sample integrating the photic zone waters, taken during the summer of 2007. NTU, nephelometric turbidity unit; DOC, dissolved organic carbon; DIN:TP, dissolved inorganic nitrogen – total phosphorus molar ratio; TN:TP, total nitrogen – total phosphorus molar ratio.

Fig. 2. Photographs of Curator Lake (*a*), McConnell Lake (*b*), and their surrounding catchment. Two rock-glacierized talus slopes flank Curator Lake, whereas recently (post-Little Ice Age) deglaciated terrain occupies the upper cirque above McConnell Lake. Photo credits: (*a*) W.O. Hobbs, August 2006, and (*b*) R.D. Vinebrooke, July 2007.



slurries were spiked with a known quantity of exotic pollen grains for calculating absolute microfossil concentration (Wolfe 1997). A range of published diatom floras were consulted for species identification (Krammer and Lange-Bertalot 1986, 1988, 1991*a*, 1991*b*; Wunsam et al. 1995; Fallu et al. 2000; Cremer et al. 2001; Moser et al. 2004), and photomicrographic documentation was conducted throughout the study using both light and scanning electron microscopy. We refer to the *Cyclotella comensis* "complex" throughout the paper, which is comprised of ecologically compatible, very small (~5 µm) morphotypes of *Cyclotella comensis* Grunow that cannot be further resolved by light microscopy. We used scanning electron microscopy to confirm the presence and defining characteristics of a singular central fultoportula and valve face rimoportula on specimens in both valve and dorsal views.

Diatom microfossils showed some signs of dissolution throughout both cores, which is common in other Canadian Rocky Mountain lakes given the low Si availability, alkaline pH, and strong microbial alteration of protective organic coatings covering diatom cells (Hobbs et al. 2010*b*). To verify that our stratigraphic trends in diatom assemblages are not being shaped by diagenetic effects, we calculated the *F* index ratio of Flower and Likhoshway (1993), which is a dissolutional index and has been rigorously tested in laboratory and field settings (Ryves et al. 2001). We then used linear regression to test whether the *F* index explained a statistically relevant portion of the trends in diatom communities.

Geochemistry

The composition of the sediments was established using loss-on-ignition (Heiri et al. 2001). Percent organic matter (% OM) was attained by combustion at 550 °C, percent carbonate-C (% CO_3) at 1000 °C, with remaining mass representing the noncarbonate inorganic fraction. Samples of sediment organic matter were analyzed for stable N isotopes ($\delta^{15}N$; ratio of ^{15}N:^{14}N as per mil (‰), relative to air (0‰)) using an elemental analyzer interfaced to a continuous flow isotope-ratio mass spectrometer at the University of California-Davis, Stable Isotope Facility. Both wet and dry deposition of N with a depleted $\delta^{15}N$ signature from fossil fuel and fertilizer NO_x emissions (including agricultural nitrification) can be assimilated by alpine lake ecosystems and recorded by lake sediments (Wolfe et al. 2003; Enders et al. 2008).

Microbial pigments

Pigments were quantified using standard reverse-phase high-pressure liquid chromatographic (HPLC) techniques specifically developed for lake sediments (Vinebrooke et al. 2002). Pigments were first extracted from freeze-dried sediments using an acetone–methanol solution. Extracts were then filtered (0.2 µm pore nylon), dried under N_2, and reconstituted using a precise volume of injection solution. Chromatographic separation was performed with an Agilent 1100 Series HPLC equipped with a Varian Microsorb 100 Å (1 Å = 0.1 nm) C18 column, and pigment detected using inline diode array and fluorescence detectors. Pigment concentrations are quantified via calibration equations and an electronic spectral library constructed using standards purchased from DHI Water and Environment (Agern Alle 5, DK-2970 Hørsholm, Denmark). HPLC chromatograms were also compared to Jeffrey et al. (2005), a key reference for taxonomically diagnostic pigments. All pigment concentrations were normalized to sediment organic matter. Owing to the potential for pigment diagenesis in this environment (Hobbs et al. 2010*b*), we have cautiously created the following summary fractions: total chlorophylls (Tchl: all primary chlorophylls and derived pheopigments) and total diatom carotenoids (TDC: fucoxanthin and diatoxanthin).

Numerical analyses

Detrended correspondence analysis (DCA) was used to summarize the dominant gradient (i.e., DCA axis 1) of diatom compositional turnover (Hill and Gauch 1980). All diatom species were included in the analysis, detrending was by segments with down-weighting of rare taxa, and nonlinear rescaling was applied. When plotted stratigraphically, the DCA axis 1 scores show the amount of turnover between samples over time in units of standard deviation (SD). However, this is not an estimate of the rate at which compositional change is occurring because the DCA is independent of sediment chronology. We determined the rate-of-change by linearly interpolating diatom percent abundance and pigment concentration data to exactly 10-year and 5-year intervals, respectively, and calculating the dissimilarity between adjacent samples (Jacobson and Grimm 1986; Lotter et al. 1992). Pigment concentration data was $\log_{10}(x + 1)$ transformed prior to dissimilarity calculations to stabilize variance within each data set. Dissimilarity was measured by Euclidean distance for the pigment data and chord distance for the diatom data, as appropriate for concentration and percent abundance data, respectively (Hall et al. 1999). The dissimilarity metrics were then divided by the amount of time between samples, giving a rate-of-change. While we do not present all sedimentary pigment data, rate-of-change calculations are based on complete chlorophyll and carotenoid results for both lakes (R.D. Vinebrooke, unpublished data). The rate-of-change of the diatom assemblage and sedimentary pigments can be interpreted as a measure of the stability of both communities through time. Interpolations were carried out using AnalySeries 2.0 (Paillard et al. 1996), while DCA and dissimilarity distances were calculated using R (R Development Core Team 2010). We express diatom species richness as the number of species per sediment interval predicted by rarefaction analysis, normalized to a count size of 400 valves ($E(S_{400})$) (Birks and Line 1992).

We used simple multiple linear regression to test relationships among biological response variables (diatom DCA axis 1, log transformed pigment concentrations, and square-root transformed diatom species abundances) and explanatory climate variables (mean annual temperature, mean winter temperature (October–May), mean maximum winter temperature (October–May), mean minimum winter temperature (October–May), mean summer temperature (June–September), mean maximum summer temperature (June–September), mean minimum summer temperature (June–September), mean winter snowfall, and spring rainfall). Instrumental data was averaged over each sediment interval. Instrumental climate data sets were acquired online from the National Climate Data and Information Archive, Environment Canada, for the Banff townsite (1888–present) and the Jasper townsite (1947–present). Both climate data sets are continuous and raw instrumental data was used in the analyses.

Results

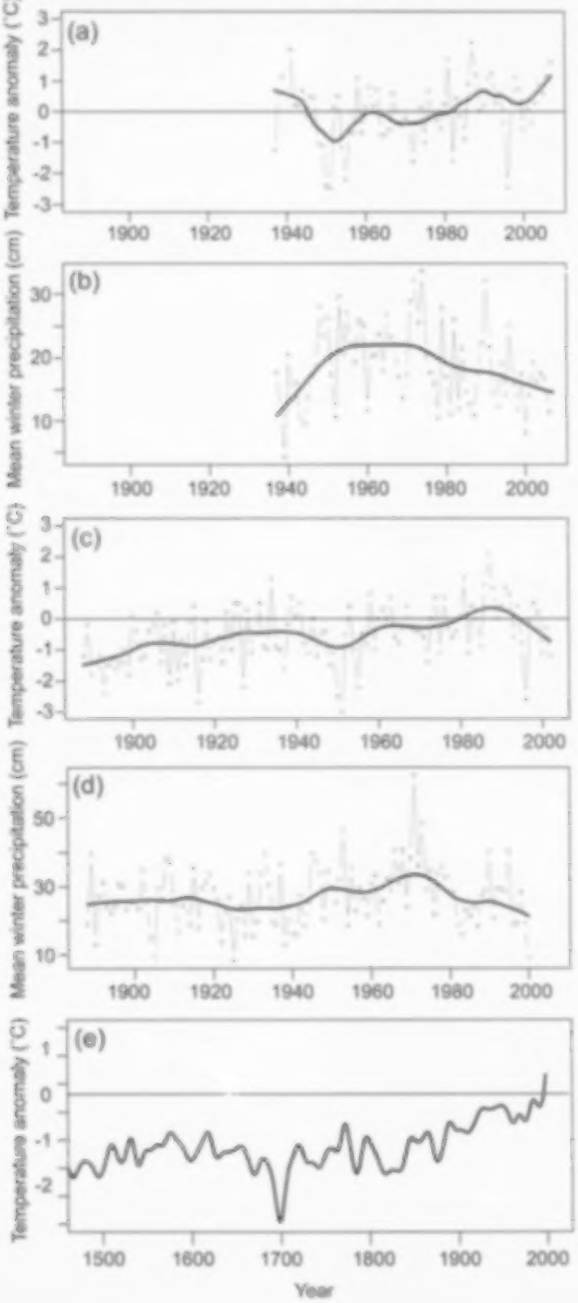
Climate records

Instrumental temperature anomalies (relative to the 1961–1990 climate normals) since 1937 for the Jasper townsite show a warm period ca. 1940 followed by cooling until 1950 and then a steady increasing trend with the post-1980 period warmer than average (Fig. 3). Winter precipitation records for Jasper are highest during the cool mid-20th century, with a decreasing trend starting in the late 1970s. At the Banff townsite, climate records extend back to 1888 and show that the 20th century is generally cooler than average with the exception of the 1980s. Similar to Jasper, the winter precipitation for Banff is greatest during the period ~1950–1970 (Fig. 3). To extend the climate record beyond the 20th century, we rely on the dendro-climatic reconstruction of Luckman and Wilson (2005), which is a compilation of records from the Banff and Jasper regions. This record shows the post-LIA warming at the end of the 19th century and through the 20th century, with post-1980s climate the warmest over the last 500 years (Fig. 3).

Core chronologies

Both sediment cores were reliably dated and generally showed slow conformable sedimentation rates over the last 200 years (Fig. 4). In Curator Lake, supported ^{210}Pb (background) was reached at a depth of 7 cm and the average dry-mass sedimentation rate for the lake is 11.7 $mg \cdot cm^{-2} \cdot year^{-1}$. McConnell Lake showed some variation in the decay of ^{210}Pb, suggesting slight sediment mixing or an increase in sedimentation, which would dilute ^{210}Pb activity (Fig. 4). We observe an increase in percent carbonate composition of the sediments associated with these variations, which suggests an increase in inorganic deposition and dilution of the ^{210}Pb activity. Therefore, we are confident the chronology established for McConnell Lake is reliable. The supported level of ^{210}Pb was reached at a depth of 10 cm and the mean dry-mass sedimentation rate is 24.5 $mg \cdot cm^{-2} \cdot year^{-1}$. Mean sedimentation rates are based on estimated sedimentation rates from the section of the core where organic content is relatively constant (below 6 cm in McConnell Lake and below 3 cm in Curator Lake). We were unable to locate any terrestrial fossils that would be appropriate for ^{14}C dating to extend our chronology beyond the ~150 year limit of ^{210}Pb dating. The uncertainties of radiocarbon dates using pollen or bulk sediment in a carbonate lake catchment are numerous (e.g., reservoir effect and low pollen abundance) and we did not consider this a viable option. We therefore employed the approach of Binford (1990) and extrapolated the ^{210}Pb model using the mean sedimentation rate. While we recognize the limitations of this method, we note that the dry bulk density ($g \cdot cm^{-3}$) has stabilized below the dated sections of both cores (McConnell Lake 495 ± 73 $mg \cdot cm^{-3}$; Curator Lake 447 ± 68 $mg \cdot cm^{-3}$).

Fig. 3. Instrumental and dendro-climatic records for the Canadian Rocky Mountains. Instrumental data from the National Climate Data and Information Archive, Environment Canada. Data presented for the Jasper townsite: (*a*) mean annual temperature, expressed as anomaly relative to the 1961–1990 normal (MAT); and (*b*) mean winter snowfall (MWS). Data for the Banff townsites shown in (*c*) MAT and (*d*) MWS. All data smoothed with a locally weighted scatterplot smoothing span of 0.2. (*e*) Dendroclimatic records from Luckman and Wilson (2005) showing the mean summer maximum temperature anomalies relative to the period 1901–1980, smoothed with a cubic spline with df = 50.



Diatom communities

We found no evidence that silica dissolution controls trends in diatom microfossil abundance or assemblage composition. No statistically significant relationships exist between the *F* index and diatom valve concentrations or species richness in either lake. Indeed, both lakes show dominance by the *Cyclotella comensis* complex, a genus that has shown relatively low resistance to dissolution (Ryves et al. 2001). We do, however, find a highly significant relationship between diatom concentrations and the primary diatom carotenoid, fucoxanthin, in Curator Lake ($r = 0.88$, $p = 1.44 \times 10^{-6}$, df = 16), suggesting that both the physical and biochemical remains of diatom assemblages are well preserved. In contrast, primary diatom pigments are absent in sediments from McConnell Lake.

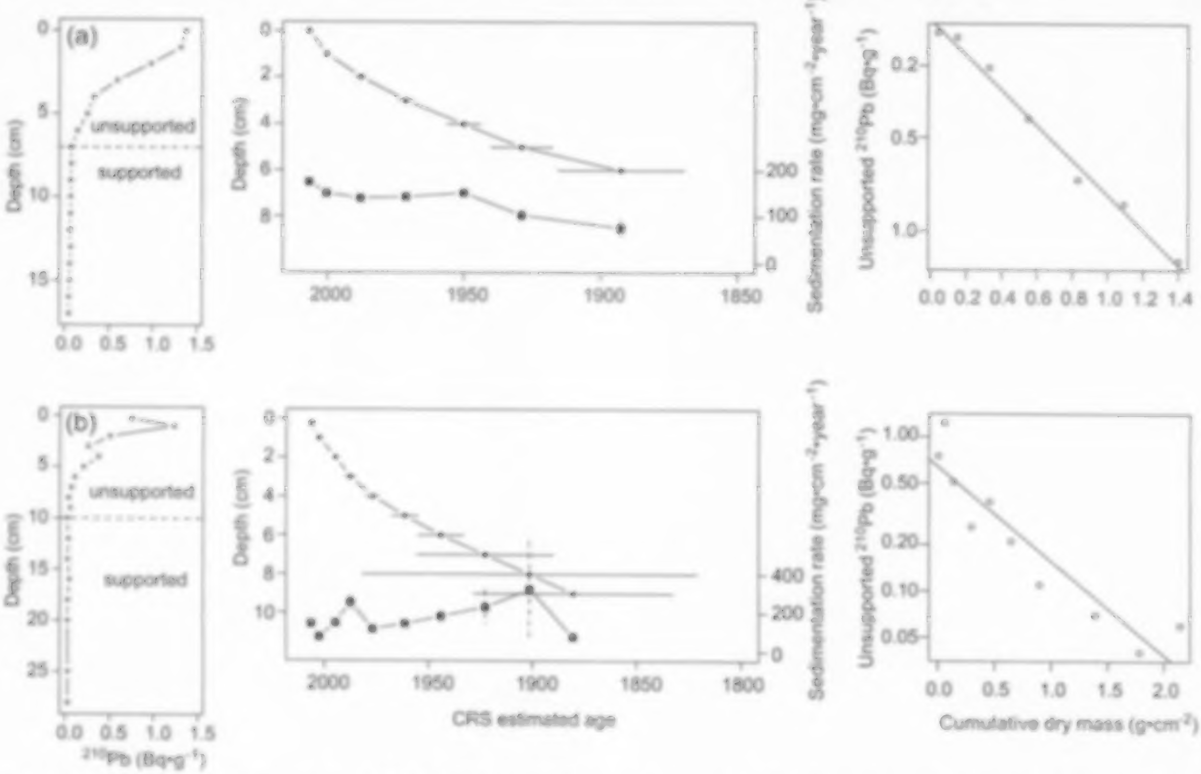
Diatom communities in both lakes are very similar in species composition and richness (Fig. 5 and Fig. 6). The dominant planktonic species are small (~5 μm) forms within the *Cyclotella comensis* complex (Fig. 7). The benthic species are dominated by colonial tychoplanktonic–benthic Fragilariaceae (*Staurosirella pinnata*, *Staurosira construens*, *Pseudostaurosira pseudoconstruens*, *Pseudostaurosira brevistriata*, and *Synedra parasitica*) (Fig. 7). In Curator Lake, minor (1%–5%) abundances of the epiphytes *Amphora pediculus*, *Amphora fogediana*, and *Gomphonema angustum* were also present with epipelic species *Eolimna minimum*, *Microcostatus kuelbsii*, *Diploneis parma*, *Navicula modica*, and *Achnanthidium minutissimum*. In McConnell Lake, minor abundances of the epiphytes *A. pediculus*, *A. fogediana*, *Gomphonema parvulum*, and *G. angustum* were present with epipilic species *Navicula cryptotenella* and *Denticula kuetzingii*.

In both lakes the DCA axis 1 scores were strong estimators of community turnover through time (Curator Lake axis 1 explained 47.8% of the variance; McConnell Lake axis 1 explained 52.7% of the variance). The axis 1 lengths were 1.41 for Curator Lake and 1.11 for McConnell Lake, suggesting linear species responses. In both lakes the community turnover is driven by the relative success of either the *Cyclotella comensis* complex or the small tychoplanktonic Fragilariaceae (Fig. 5 and Fig. 6). In Curator Lake the *C. comensis* complex is strongly dominant (>50% abundance) during the recent period of 1980–present, whereas it is subdominant (20%–35%) during the periods 1480–1510 and 1630–1850. The small Fragilariaceae group is subdominant during the same time periods and strongly dominant during the periods 1510–1630 and 1850–1980. In McConnell Lake the *C. comensis* complex is strongly dominant for shorter time periods 1580–1600, 1680–1750, and 1910–1965, whereas the Fragilariaceae group is strongly dominant during the intervening time periods. The trends of planktonic and benthic dominance are summarized by the planktonic:benthic ratio for the two lakes (Fig. 5 and Fig. 6). The DCA axis 1 scores show that the turnover of the diatom communities over time is gradual in Curator Lake (Fig. 5), whereas the McConnell Lake communities shifted more abruptly (Fig. 6).

Microbial pigments

Large increases in photoautotrophic production during the 20th century are recorded in the total chlorophyll content of the sediments from both lakes (Fig. 5 and Fig. 6). In Curator Lake this increase in production is mainly present as chromo-

Fig. 4. Results of ^{210}Pb dating for (*a*) Curator and (*b*) McConnell lakes. Graphs, from left to right, show the total ^{210}Pb inventories with depth, highlighting the depth of background activities. The middle graph shows the modeled sediment ages (small black circles) and calculated sedimentation rates (small black squares) with ±1 SD error bars. The graphs to the right show the linear relationships between unsupported ^{210}Pb and cumulative dry mass, suggesting fairly conformable sedimentation towards the limit of ^{210}Pb dating.



phytes (chrysophytes, diatoms, and dinoflagellates), which is also shown by the consistent trend in TDC (Fig. 5). The trend in McConnell Lake is more variable over the length of the core (Fig. 6). Vinebrooke et al. (2010) have characterized this increase in primary production as mainly due to deep-water bacteria, including planktonic green and purple sulfur groups, and picocyanobacteria (recorded as zeaxanthin). The TDC profile in McConnell Lake is possibly affected by diagenesis, given the lack of correlation with the diatom valve abundance (Fig. 6).

Sediment composition and geochemistry

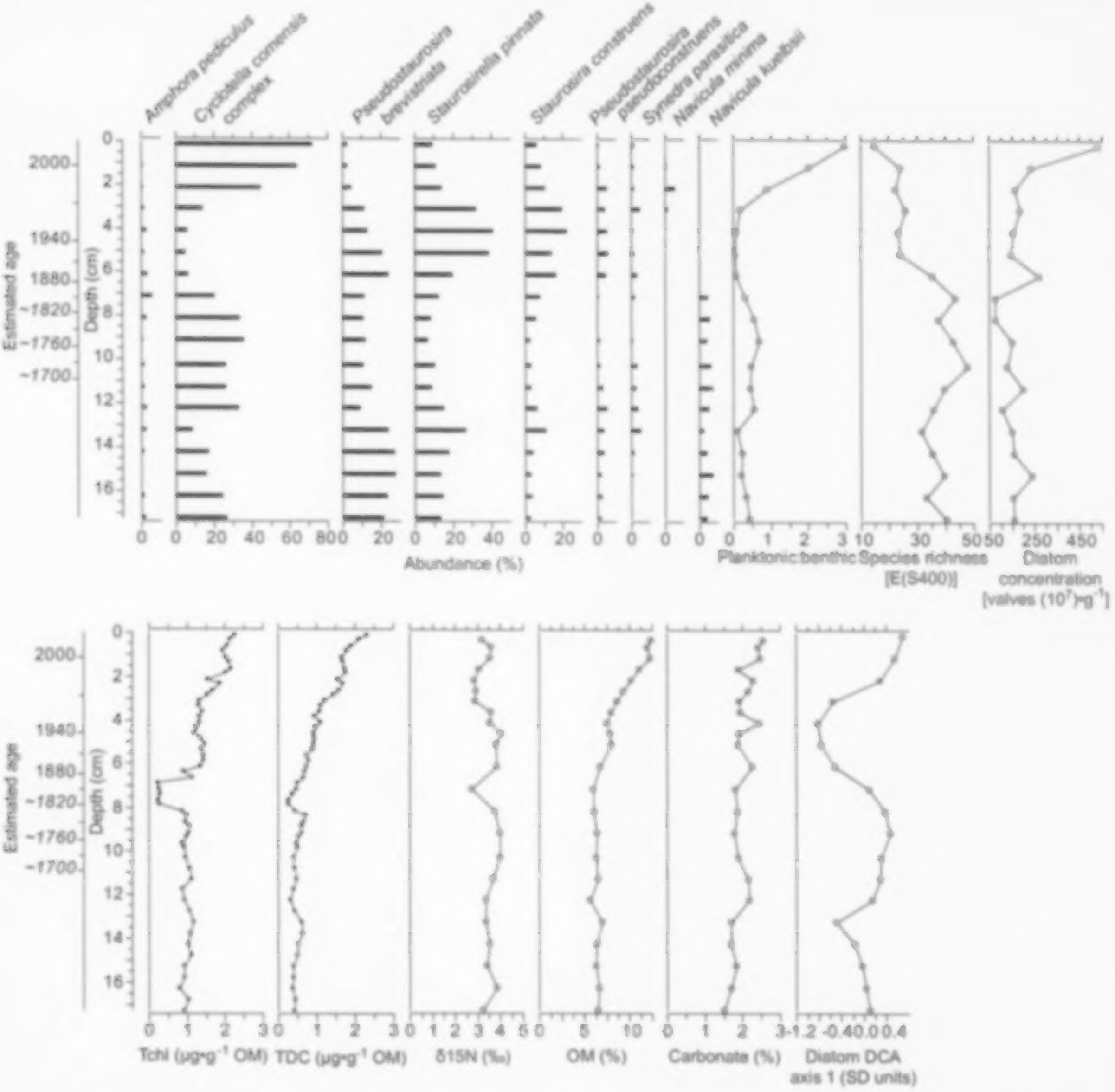
Sedimentological properties of the cores are somewhat similar between the cores. In both lakes the majority of the sediment is comprised of an inorganic fraction (Curator Lake 85%–92%; McConnell Lake 76%–90%). Both lakes record an increase in % OM during the 20th century, after ~1930 in Curator Lake (Fig. 5) and after ~1910 in McConnell Lake (Fig. 6). Curator Lake ranges from 6% to 13% with a pre-1930 background of 6.4% ± 0.3%, whereas McConnell Lake ranges from 3% to 12% with a pre-1910 background of 4.3% ± 0.5%. Carbonate abundance in Curator Lake is low and varies very little throughout the core (2.0% ± 0.3%), whereas McConnell Lake ranges from 9% to 18% with a mean of 12.2% ± 2.5%.

The $\delta^{15}N$ of sediment organic matter in Curator Lake varies very little throughout the core with a mean of 3.46‰ ± 0.39‰ (Fig. 5). McConnell Lake records a very similar $\delta^{15}N$ for the pre-1910 section of the core (3.48‰ ± 0.57‰) (Fig. 6). Following 1910, $\delta^{15}N$ is depleted, but remains relatively stable (2.21‰ ± 0.21‰) until 1970 when a significant monotonic depletion takes place from 2.61 ‰ to 0.65 ‰ (Fig. 6). This recent isotopic depletion is directly comparable to sediment records from lakes influenced by atmospheric deposition (Wolfe et al. 2003; Enders et al. 2008).

Biological rate of change

The rate-of-change in diatom communities and sedimentary algal pigments is greatest during the 20th century in the context of the last ~500 years (Fig. 8). In Curator Lake, the rate at which diatom communities change reflects the shifts between planktonic and benthic species, with the greatest change happening during the periods 1600–1650, 1850–1910, and 1980 to present. The rate at which all algal groups (as Tchl) changed is similar to the response of diatoms alone, reflecting the importance of diatoms in the whole-lake primary production in this lake. The pigment rate-of-change also shows much greater short-term variability following ~1930 (Fig. 8). In McConnell Lake, the diatom and pigment rate-of-change trends are different, supporting the observation that diatoms do not dominate the whole-lake primary production (Fig. 8). The trends in McConnell Lake are also much

Fig. 5. Biogeochemical stratigraphy of Curator Lake. Relative frequencies of dominant diatoms shown with absolute diatom concentrations and rarefaction-estimated species richness [E(S_{400})]. The second row illustrates total diatom carotenoids (TDC), total chlorophylls (Tchl), δ^{15}N, sediment composition (percent organic matter (% OM) and percent carbonate), and diatom community turnover as summarized by DCA axis 1 sample scores.



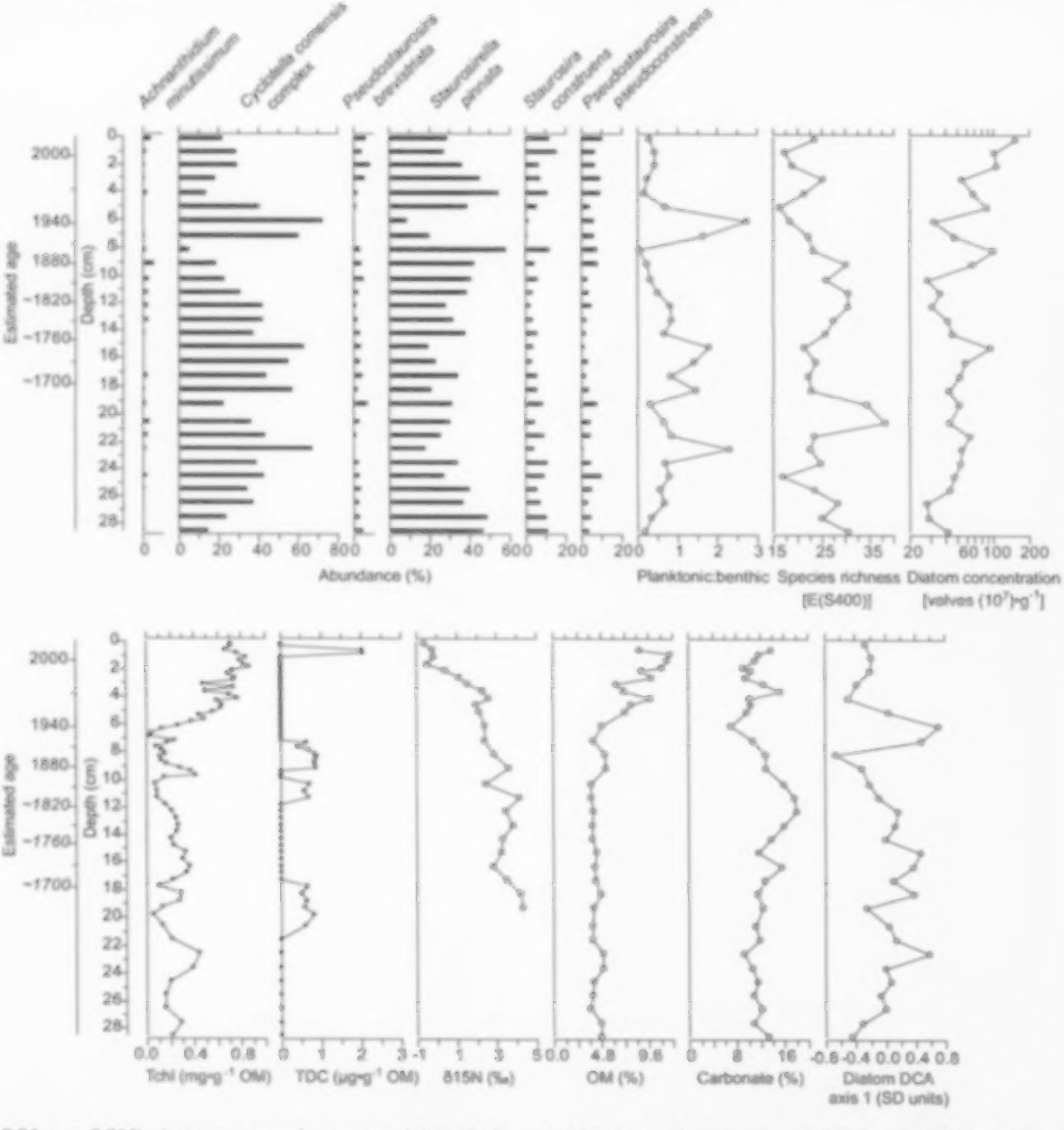
more variable over the length of the record. The greatest rate-of-change recorded in the diatom communities occurs following 1910; whereas, in the pigment record the greatest rate-of-change is within the last 20 years.

Discussion

Climatic drivers of ecological change

Profound ecological changes have occurred in the studied alpine lakes during the 20th century. Although similar shifts in photoautotrophic communities have occurred in the past, the changes in the last century are unique in both magnitude and rate, in the context of the last ~500 years. Ecological shifts in mountain lakes of the Canadian Rocky Mountains have been previously attributed to changing climate (Karst-Riddoch et al. 2005; Parker et al. 2008). We see evidence of a direct influence by climate during the 20th century on the diatom assemblage turnover of Curator Lake (Fig. 9*a*). We found a strong linear correlation between the instrumental annual mean temperature record and diatom DCA axis 1 (*r* =

Fig. 6. Biogeochemical stratigraphy of McConnell Lake. Relative frequencies of dominant diatoms shown with absolute diatom concentrations and rarefaction-estimated species richness [E(S_{400})]. The second row illustrates total diatom carotenoids (TDC), total chlorophylls (Tchl), $\delta^{15}N$, sediment composition (percent organic matter (% OM) and percent carbonate), and diatom community turnover as summarized by DCA axis 1 sample scores.



0.94; $p = 0.016$). As temperatures have warmed through the 20th century we find a positive response from the planktonic *Cyclotella comensis* complex ($r = 0.94$; $p = 0.019$) and a strong negative response by the small colonial Fragilariaceae ($r = -0.93$; $p = 0.022$). The proposed mechanism for this response is increasing thermal stratification (and possibly earlier onset) with warmer air temperatures, which favors the habitat of small *Cyclotella* species that exploit the thermocline (Rühland et al. 2008; Winder et al. 2009). This strong response to the length of ice-cover by *Staurosirella pinnata*, *Staurosira construens*, and *Cyclotella* species has also been recorded in seasonal sediment trap sampling in the Swiss Alps, Switzerland (Lotter and Bigler 2000). We find a significant linear correlation between annual mean temperature and

Fig. 7. Scanning electron microscopy and light microscopy micrographs of dominant diatom taxa found in both lakes: *Cyclotella comensis* complex (*a*–*q*) and small colonial Fragilariaceae species (*r*–*c*′). *Cyclotella* scale bars are 5 µm and Fragilariaceae are 2 µm. (*a*–*i*). Scanning electron microscopy images of *Cyclotella comensis* morphotypes showing central fultoportula(e) (cfp); marginal fultoportulae (mfp); and rimoportula(e) (rp). The valve face morphology ranges from circumferentially undulate (*d* and *g*) to radial undulations (*b*) to a relatively smooth face (*h*). Micrograph (*g*) is the sole *Cyclotella comensis*, while the remainder of the specimens in scanning electron microscopy are *C.* cf. *comensis*. A singular cfp and rp are evident in the dorsal views of the *C. comensis* complex, a defining characteristic of this genus. In light microscopy, variations in valve morphology are indistinguishable (*j*–*q*). The larger specimen (*j*) can be considered closer to *C. pseudocomenesis*. Individuals of small colonial Fragilariaceae (*Fragilaria* s.l.) include: *Staurosirella pinnata* in valve view (*r*), dorsal view (*s*, *t*, *v*, and *w*), and girdle view (*u*); *Staurosira construens* var. *venter* in valve view (*x*), dorsal view (*y*), and girdle view (*z*); *Pseudostaurosira pseudoconstruens* in dorsal view (*a*′ and *b*′); *Pseudostaurosira brevistriata* in dorsal view (*c*′). Interior views of these taxa all reveal the presence of apical pore fields.

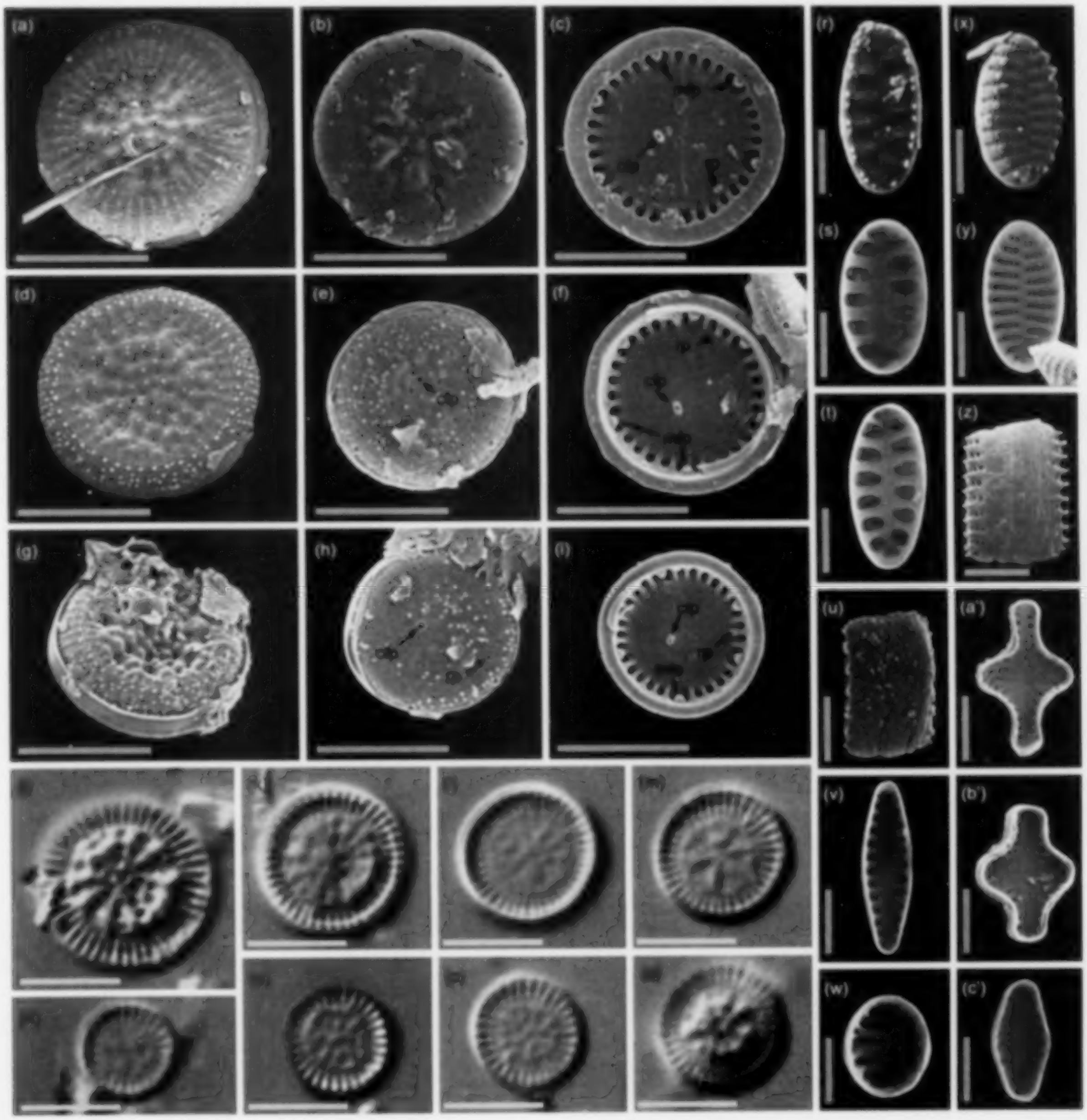


Fig. 8. Rate-of-change in diatom (black line) and pigment (grey line) assemblages over the last ~500 years for Curator Lake (*a*) and McConnell Lake (*b*).

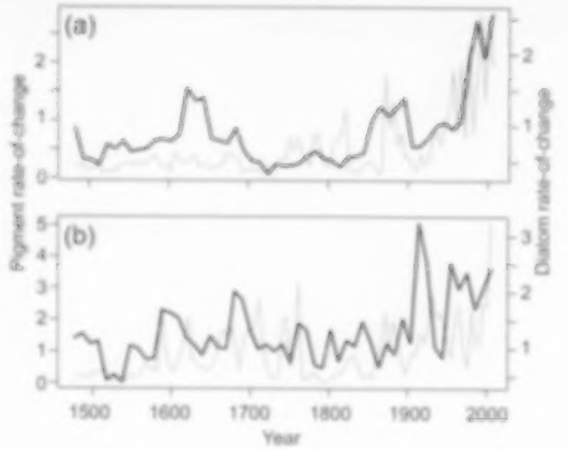
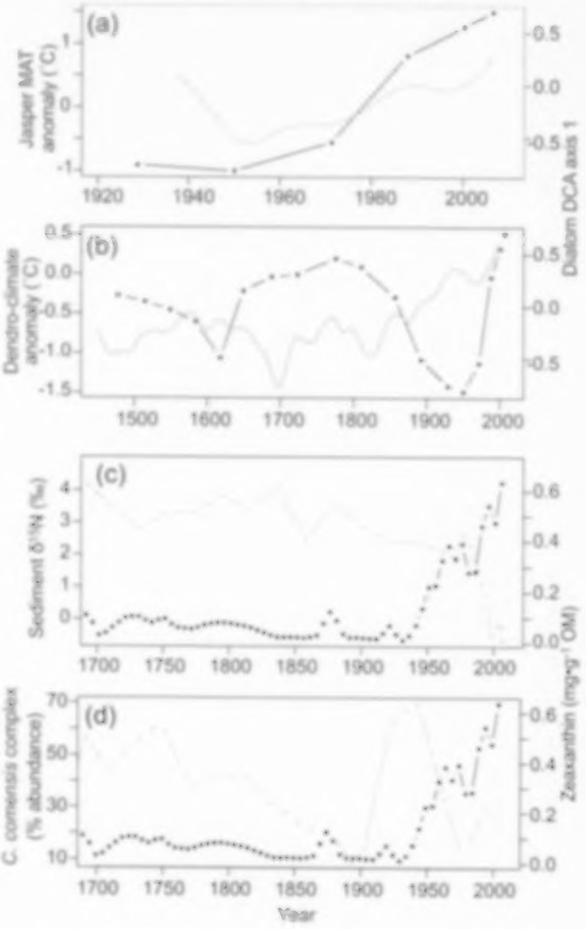


Fig. 9. Summary of relationships between abiotic drivers and algal responses. (*a*) Jasper MAT (grey line) and Curator Lake diatom community turnover (black line: DCA axis 1 scores). (*b*) Dendroclimatic summer maximum temperature anomalies (grey line) and Curator Lake diatom turnover (black line). (*c*) Sedimentary $\delta^{15}N$ record (grey line) in McConnell Lake and zeaxanthin concentrations (black line) attributed to picocyanobacteria. (*d*) Zeaxanthin (black line) and the *Cyclotella comensis* complex (grey line) in McConnell Lake provide evidence of emerging competition between picocyanobacteria and planktonic diatoms during the 20th century.



sediment Tchl ($r = 0.52$; $p = 0.027$), which has also been shown by Vinebrooke et al. (2010) to correlate with dendroclimatic reconstructions (Luckman and Wilson 2005) spanning the length of the core. We were unable to find further correlations with the regional dendroclimatic record (Fig. 9*b*). The activity of the nearby Cavell and Centre Glaciers during the LIA suggest that colder temperatures prevailed in the mid-17th, early 18th, and mid-19th centuries (Luckman 2000). However, given the complacent sedimentology of our core (% OM and percent carbonate), there appears to have been little glacial activity directly impacting Curator Lake and the catchment. The rate-of-change in the aquatic ecosystem of Curator Lake shows little change during the colder periods of regional glacial activity, with periods of instability that correlate with the onset (early 1600s) and subsidence (late 1800s) of the LIA. The late 20th century change is considerably greater and more variable than that observed during the LIA, suggesting the temperature during this period has perhaps elicited a threshold response. Based on the abundance of the *Cyclotella comensis* complex, which represents increased temperatures and a stratified water column, the marked post-1980 increase suggests climate warming at Curator Lake is beyond any interval of the prior ~500 years.

Relative to Curator Lake, the climate signal expressed at McConnell Lake is more closely mediated by catchment processes, in particular those associated with the headwall glacier. Shifts in the diatom assemblages suggest an indirect link to climate, through the influence of glacial activity and hydrology within the catchment, however there are no significant relationships between sediment biological proxies and instrumental or dendroclimatic records. The dramatic shift from a benthic dominated assemblage to the *Cyclotella comensis* complex at ~1910 coincides with a distinct change in sedimentology (increased organic content) and enhanced whole-lake primary production. This shift is coincident with reduced glacial inputs (rock flour), which likely caused associated decreases in lake turbidity and increased light penetration, stimulating primary production. Prior to this shift (late 19th century) the sedimentation rate in McConnell Lake increases, a response that has been associated with glacial recession during a similar time period in the nearby Hector Lake (Leonard 1997). The maximum % CO_3 in the McConnell Lake core coincides with the LIA maximum during ~1830–1860 for this region (Luckman 2000), supporting the clear influence of glacial activity on sedimentation in McConnell Lake and suggesting a similar timing of the LIA maximum in the basin. The regional LIA maximum also coincides with a reduction in the *C. comensis* complex and strong dominance by *Staurosirella pinnata*, and some of the lowest whole-lake primary production of the last 500 years in McConnell Lake. Together the response of the diatom communities and primary production shows that the aquatic ecosystem of McConnell Lake is closely tied to the surrounding catchment.

The post-1910 section of the McConnell Lake core records an increase in organic sediment as the glaciers ablate. The increase in primary production is attributable to sulfur-reducing phototrophic bacteria (recorded as monomers of bacteriochlorophylls), picocyanobacteria (recorded by zeaxanthin concentrations), and planktonic diatoms. The dominance by the *C. comensis* complex lasts ~60 years (until 1970), at which time the benthic communities become dominant. With increased clarity of the water column and strong summer stratification, it is possible that benthic growth has been stimulated by an expansion of the lake's photic zone. Another plausible mechanism is that phototrophic bacteria blooms are out-competing planktonic diatoms for light and nutrients (Fig. 9*d*). *Cyclotella comensis* require a strongly stratified water column, which also provides an ideal habitat for deepwater sulfur-reducing phototrophic bacteria that thrive if the photosynthetic compensation depth extends well into anoxic hypolimnion. Because both may bloom in the late summer in alpine lakes (Lotter and Bigler 2000; Lafrançois et al. 2003), intense competition for resources is predicted. Experimental mesocosms have shown that phototrophic bacterial responses in alpine lakes are stimulated by increased Nr concentrations (Lafrançois et al. 2003; Nydick et al. 2004), whereas *C. comensis* is only a strong competitor for Nr during low N:P conditions (Winder et al. 2009). We therefore need to consider in detail the influence of changing Nr inputs to McConnell Lake during the post-1970 period, when *C. comensis* abundance is low.

N cycling, atmospheric Nr deposition, and nutrient limitation

Increases in atmospheric anthropogenic Nr have been recorded by deviations in N stable isotopic ratios from lake sediments (Wolfe et al. 2003), tree-rings (Savard et al. 2009), and Greenland ice cores (Hastings et al. 2009). In lake sediments, overprinting of the primary isotopic signal by organic matter diagenesis is a constant concern (Peterson and Fry 1987; Gälman et al. 2009), although, these processes are typically of lesser magnitude than the isotopic signature of source Nr. It is also noted that the principal diagenetic processes in lake sediments generally exploit ^{14}N preferentially, leaving the substrate enriched with respect to ^{15}N. This is the opposite of trends observed in association with anthropogenic Nr sources (Enders et al. 2008). We also note that changes in the sparse catchment vegetation should not exert a significant influence on the $\delta^{15}N$ of dissolved N within either lake, similar to findings of other lakes above tree line in the Canadian Rocky Mountains (Bunting et al. 2010).

Although both Curator and McConnell lakes produce comparable background sediment $\delta^{15}N$ (~3.5‰), it is only in the McConnell Lake sequence that recent rapid declines in sediment isotopic values are observed, in the order of 3.0‰. Both lakes are likely to receive Nr and other pollutants from regional sources including transportation (the TransCanada Highway and Icefields Parkway), industrial processes (cement production in the Bow River valley), and historically coal mining. McConnell Lake is the more proximal to these sources, and here sediment $\delta^{15}N$ begins to decline directionally after 1910, followed by a dramatic isotopic depletion post-1970. This record contains two important elements concerning N cycling, which much be considered with the realization that the $\delta^{15}N$ profile from Curator Lake, ~200 km to the north, does not vary significantly over the last ~500 years; we infer that no dramatic change in Nr source or cycling has occurred in Curator Lake despite climate-driven changes in primary production and diatom composition. First, the McConnell Lake $\delta^{15}N$ profile parallels the course of ablation of the catchment's glaciers closely, suggesting that Nr archived in glacier ice is relegated to surface waters upon melt. It has been established that glacially influenced lakes have consistently higher NO_3^- concentrations relative to nearby nonglacial counterparts (Saros et al. 2010). This partially explains the absence of any changes in sediment $\delta^{15}N$ of Curator Lake. Secondly, the interval of greatest sediment $\delta^{15}N$ depletion in McConnell Lake occurs after 1970 (~3‰), coinciding closely with increased picocyanobacterial production recorded as zeaxanthin (Fig. 9). Increased picocyanobacteria production is likely being driven by greater Nr inputs and thermal stratification. Because cyanobacterial metabolism fractionates very little, increased representation of this Nr source in sediment organic matter will likely reduce bulk sediment $\delta^{15}N$ (Peterson and Fry 1987). This is another difference with Curator Lake, for which cyanobacteria are not a substantial component of the algal community.

High Nr deposition in the southern Rocky Mountains has been implicated in shifting nutrient limitation of phytoplankton from N to P (Elser et al. 2009*b*). Water chemistry and evidence from nutrient bioassays reveals strong P limitation in both Curator and McConnell lakes (Murphy et al. 2010), but the stability of the Curator Lake $\delta^{15}N$ record suggests this has been the prevailing resource limitation for the last ~500 years. This is because the sudden alleviation of N limitation should result in a measurable $\delta^{15}N$ response as the ability of algae to discriminate against ^{15}N increases (Teranes and Bernasconi 2000). Because the post-1910 isotopic depletion observed in McConnell Lake sediment may be attributed to the release of legacy Nr from melting snow and ice, it is likely that P limitation has intensified in this lake. Increased Nr availability that induced wholesale shifts in phytoplankton dominance patterns, is likely the primary control over the McConnell Lake $\delta^{15}N$ stratigraphy, indicating profound shifts in N cycling. The absence of these processes in Curator Lake, which has nonetheless changed markedly in other ways, illustrates how differential responses may arise in superficially similar lake ecosystems subjected to the same suite of stressors.

Mediating effects of the lake catchment

Sediments from Curator and McConnell lakes preserve evidence for a range of ecosystem responses during recent centuries, which we feel are strongly mediated by lake catchment processes. Although the lakes studied are similar with respect to size, basin morphology, and water chemistry, they have been variably influenced by similar abiotic drivers. Major shifts are temporally asynchronous and expressed differentially among photoautotrophic groups (e.g., diatoms and phototrophic bacteria). These findings highlight how context-dependent the response of alpine lakes to anthropogenic forcing can be. Despite these complexities, there is overwhelming evidence supporting the notion of accelerated environmental changes since the mid-20th century, in keeping with the general trend observed across remote lakes of the northern hemisphere (Hobbs et al. 2010*a*). It remains unclear exactly what future ecological trajectories these lakes will follow. Future climate warming, in many cases associated with the reduction of hydrological inputs from snow and ice, will likely create positive feedbacks involving increased primary production and organic matter supply to sediments. It is unclear what continued biological turnover will mean for the upper trophic levels and the health of the aquatic ecosystem as climate warming continues unabated.

Acknowledgements

The authors thank Erin Doxsey-Whitfield and Suzanne McGowan for assistance in the field and Colin Cooke, Mark Graham, Patrick Thompson, David Harris, and Collin Quarrie for laboratory assistance. The authors thank three anonymous reviewers for constructive comments that improved this manuscript. Funding was provided by the Alberta Water Research Institute and the Natural Sciences and Engineering Research Council of Canada (NSERC).

References

Appleby, P.G. 2010. Chronostragraphic techniques in recent sediments. *In* Tracking environmental change using lake sediments. Basin analysis, coring, and chronological techniques. Vol 1. *Edited by* W.M. Last and J.P. Smol. Kluwer Academic, Dordrecht, the Netherlands. pp. 171–203.

Appleby, P.G., and Oldfield, F. 1978. The calculation of lead–210 dates assuming a constant rate of supply of unsupported ^{210}Pb to the sediment. Catena, **5**(1): 1–8. doi:10.1016/S0341-8162(78)80002-2.

Battarbee, R.W., Jones, V.J., Flower, R.J., Cameron, N.G., Bennion, H., Carvalho, L., and Juggins, S. 2001. Diatoms. *In* Tracking environmental change using lake sediments. Terrestrial, algal, and siliceous indicators. Vol. 3. *Edited by* J.P. Smol, H.J.B. Birks, and W.M. Last. Kluwer Academic Publishers, Dortrecht, the Netherlands. pp. 155–202.

Bergström, A.K., and Jansson, M. 2006. Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. Glob. Change Biol. **12**(4): 635–643. doi:10.1111/j.1365-2486.2006.01129.x.

Binford, M. 1990. Calculation and uncertainty analysis of Pb 210 dates for PIRLA project lake sediment cores. J. Paleolimnol. **3**(3): 253–267. doi:10.1007/BF00219461.

Birks, H.J.B., and Line, J.M. 1992. The use of rarefaction analysis for estimating palynological richness from Quaternary pollen-analytical data. Holocene, **2**: 1–10.

Bunting, L., Leavitt, P.R., Weidman, R.P., and Vinebrooke, R.D. 2010. Regulation of the nitrogen biogeochemistry of mountain lakes by subsidies of terrestrial dissolved organic matter and the implications for climate studies. Limnol. Oceanogr. **55**(1): 333–345. doi:10.4319/lo.2010.55.1.0333.

Cremer, H., Wagner, B., Melles, M., and Hubberten, H.W. 2001. The postglacial environmental development of Raffles Sø, East Greenland: inferences from a 10,000 year diatom record. J. Paleolimnol. **26**(1): 67–87. doi:10.1023/A:1011179321529.

Davidson, E.A. 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. Nat. Geosci. **2**(9): 659–662. doi:10.1038/ngeo608.

Desloges, J.R., and Gilbert, R. 1995. The sedimentary record of Moose Lake: implications for late-glacial and Little Ice Age glacier activity in the Mount Robson Area, British Columbia. Can. J. Earth Sci. **32**: 65–78.

Elser, J.J., Kyle, M., Steger, L., Nydick, K.R., and Baron, J.S. 2009*a*. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology, **90**(11): 3062–3073. doi:10.1890/08-1742.1. PMID:19967862.

Elser, J.J., Andersen, J., Baron, J.S., Bergström, A., Jansson, M., Kyle, M., Nydick, K.R., Steger, L., and Hessen, D. 2009*b*. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science, **326**(5954): 835–837. doi:10.1126/science.1176199. PMID:19892979.

Enders, S., Pagani, M., Pantoja, S., Baron, J.S., Wolfe, A.P., Pedentchouk, N., and Nuñez, L. 2008. Compound-specific stable isotopes of organic compounds from lake sediments track recent environmental changes in an alpine ecosystem, Rocky Mountain National Park, Colorado. Limnol. Oceanogr. **53**(4): 1468–1478. doi:10.4319/lo.2008.53.4.1468.

Falkowski, P., Scholes, R.J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Högberg, P., Linder, S., Mackenzie , F.T., Moore, B., Pedersen, T., Rosenthal, Y., Seitzinger, S., Smetacek, V., and Steffen, W. 2000. The global carbon cycle: a test of our knowledge of earth as a system. Science, **290**(5490): 291–296. doi:10.1126/science.290.5490.291. PMID:11030643.

Fallu, M.A., Allaire, N., and Pienitz, R. 2000. Freshwater Diatoms from northern Québec and Labrador (Canada): species–environment relationships in lakes of boreal forest, forest–tundra and tundra regions. Bibliotheca Diatomologica Band 45. E. Schweizerbart, Germany.

Flower, R., and Likhoshway, Y. 1993. An investigation of diatom preservation in Lake Baikal. *In* Fifth Workshop on Diatom Algae. *Edited by* M.A. Grachev. Russian Botanical Society, Irkutsk, Russia. pp. 77–78.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., and Vörösmarty, V.J. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry, **70**: 153–226. doi:10.1007/s10533-004-0370-0.

Gälman, V., Rydberg, J., and Bigler, C. 2009. Decadal diagenetic effects on δ^{13}C and δ^{15}N studied in varved lake sediment. Limnol. Oceanogr. **54**(3): 917–924. doi:10.4319/lo.2009.54.3.0917.

Glew, J.R., Smol, J.P., and Last, W.M. 2001. Sediment core collection and extrusion. *In* Tracking environmental change using lake sediments. Basin analysis, coring, and chronological techniques. Vol. 1. *Edited by* W.M. Last and J.P. Smol. Kluwer Academic Publishers, Dordrecht, the Netherlands. pp. 73–106.

Hall, R., Leavitt, P.R., Quinlan, R., Dixit, A., and Smol, J.P. 1999. Effects of agriculture, urbanization, and climate on water quality in the northern Great Plains. Limnol. Oceanogr. **44**(3, part 2): 739–756. doi:10.4319/lo.1999.44.3_part_2.0739.

Hastings, M.G., Jarvis, J.C., and Steig, E.G. 2009. Anthropogenic impacts on nitrogen isotopes of ice-core nitrate. Science, **324**(5932): 1288. doi:10.1126/science.1170510. PMID:19498161.

Heiri, O., Lotter, A.F., and Lemcke, G. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. J. Paleolimnol. **25**(1): 101–110. doi:10.1023/A:1008119611481.

Hill, M.O., and Gauch, H.G., Jr. 1980. Detrended correspondence-analysis — an improved ordination technique. Vegetatio, **42**(1–3): 47–58. doi:10.1007/BF00048870.

Hobbs, W.O., Telford, R.J., Birks, H.J.B., Saros, J.E., Hazewinkel, R.R.O., Perren, B.B., Saulnier-Talbot, É., and Wolfe, A.P. 2010*a*. Quantifying recent ecological changes in remote lakes of North

America and Greenland using sediment diatom assemblages. PLoS ONE, **5**(4): e10026. doi:10.1371/journal.pone.0010026. PMID:20368811.

Hobbs, W.O., Lalonde, S.V., Vinebrooke, R.D., Konhauser, K.O., Weidman, R.P., Graham, M.D., and Wolfe, A.P. 2010*b*. Algal-silica cycling and pigment diagenesis in recent alpine lake sediments: mechanisms and paleoecological implications. J. Paleolimnol. **44**(2): 613–628. doi:10.1007/s10933-010-9441-5.

Jacobson, G.L., and Grimm, E.C. 1986. A numerical analysis of Holocene forest and prairie vegetation in central Minnesota. Ecology, **67**(4): 958–966. doi:10.2307/1939818.

Jeffrey, S.W., Mantoura, R.F.C., and Wright, S.W. 2005. Phytoplankton pigments in oceanography: guidelines to modern methods. 2nd ed. UNESCO Publishing, Paris, France.

Karst-Riddoch, T.L., Pisaric, M.F.J., and Smol, J.P.S. 2005. Diatom responses to 20th century climate-related environmental changes in high-elevation mountain lakes of the northern Canadian Cordillera. J. Paleolimnol. **33**(3): 265–282. doi:10.1007/s10933-004-5334-9.

Krammer, K., and Lange-Bertalot, H. 1986. Bacillariophyceae. 1. Teil: Naviculaceae. *In* Süßwasserflora von Mitteleuropa. *Edited by* H. Ettl, G. Gärtner, J. Gerloff, H. Heynig, and D. Mollenhauer. Band 2/1, Gustav Fischer Verlag, Stuttgart, Germany/New York, N.Y.

Krammer, K., and Lange-Bertalot, H. 1988. Bacillariophyceae. 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. *In* Süßwasserflora von Mitteleuropa. *Edited by* H. Ettl, G. Gärtner, J. Gerloff, H. Heynig, and D. Mollenhauer. Band 2/2, Gustav Fischer Verlag, Stuttgart, Germany/New York, N.Y.

Krammer, K., and Lange-Bertalot, H. 1991*a*. Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. *In* Süßwasserflora von Mitteleuropa. *Edited by* H. Ettl, G. Gärtner, J. Gerloff, H. Heynig, and D. Mollenhauer. Band 2/3, Gustav Fischer Verlag, Stuttgart/Jena, Germany.

Krammer, K., and Lange-Bertalot, H. 1991*b*. Bacillariophyceae. 4. Teil: Achnanthaceae Kritische Ergäzungen zu Navicula (Lineolatae) und Gomphonema. *In* Süßwasserflora von Mitteleuropa. *Edited by* H. Ettl, G. Gärtner, J. Gerloff, H. Heynig, and D. Mollenhauer. Band 2/4, Gustav Fischer Verlag, Stuttgart/Jena, Germany.

Lafrançois, B.M., Nydick, K.R., and Caruso, B. 2003. Influence of nitrogen on phytoplankton biomass and community composition in fifteen Snowy Range Lakes (Wyoming, USA). Arct. Antarct. Alp. Res. **35**(4): 499–508. doi:10.1657/1523-0430(2003)035[0499:IONOPB]2.0.CO;2.

Landers, D.H., Simonich, S.L., Jaffe, D.A., Geiser, L.H., Campbell, D.H., Schwindt, A.R., Schreck, C.B., Kent, M.L., Hafner, W.D., Taylor, H.E., Hageman, K.J., Usenko, S., Ackerman, L.K., Schrlau, J.E., Rose, N.L., Blett, T.F., and Erway, M.M. 2008. The Fate, Transport, and Ecological Impacts of Airborne Contaminants in Western National Parks (USA). EPA/600/R-07/138. US Environmental Protection Agency, Office of Research and Development, NHEERL, Western Ecology Division, Corvallis, Ore.

Last, W.M., and Smol, J.P. (*Editors*). 2001 Tracking environmental change using lake sediments, volume 2: physical and geochemical methods. Kluwer Academic Publishers, Dortrecht, the Netherlands.

Leonard, E.M. 1997. The relationship between glacial activity and sediment production: evidence from a 4450-year varve record of Neoglacial sedimentation in Hector Lake, Alberta. J. Paleolimnol. **17**(3): 319–330. doi:10.1023/A:1007948327654.

Lotter, A.F., and Bigler, C. 2000. Do diatoms in the Swiss Alps reflect the length of ice-cover? Aquat. Sci. **62**(2): 125–141. doi:10.1007/s000270050002.

Lotter, A.F., Ammann, B., and Sturm, M. 1992. Rates of change and chronological problems during the late-glacial period. Clim. Dyn. **6**(3–4): 233–239. doi:10.1007/BF00193536.

Luckman, B.H. 2000. The Little Ice Age in the Canadian Rockies. Geomorphology, **32**(3–4): 357–384. doi:10.1016/S0169-555X(99)00104-X.

Luckman, B.H., and Wilson, R.J.S. 2005. Summer temperatures in the Canadian Rockies during the last millennium: a revised record. Clim. Dyn. **24**(2–3): 131–144. doi:10.1007/s00382-004-0511-0.

Moser, K.A., Smol, J.P., and MacDonald, G.M. 2004. Ecology and distribution of diatoms from boreal forest lakes in Wood Buffalo National Park, Northern Alberta and the Northwest Territories, Canada. Academy of Natural Sciences, Philadelphia, Pa. No. 22.

Murphy, C.A., Thompson, P.L., and Vinebrooke, R.D. 2010. Assessing the sensitivity of alpine lakes and ponds to nitrogen deposition in the Canadian Rocky Mountains. Hydrobiologia, **648**(1): 83–90. doi:10.1007/s10750-010-0146-6.

Nydick, K.R., Lafrançois, B.M., Baron, J.S., and Johnson, B. 2004. Nitrogen regulation of algal biomass, productivity, and composition in shallow mountain lakes, Snowy Range, Wyoming, USA. Can. J. Fish. Aquat. Sci. **61**(7): 1256–1268. doi:10.1139/f04-085.

Paillard, D., Labeyrie, L., and Yiou, P. 1996. Macintosh program performs time-series analysis. Eos Trans. AGU, **77**(39): 379. doi:10.1029/96EO00259.

Parker, B.R., Vinebrooke, R.D., and Schindler, D.W. 2008. Recent climate extremes alter alpine lake ecosystems. Proc. Natl. Acad. Sci. U.S.A. **105**(35): 12927–12931. doi:10.1073/pnas.0806481105. PMID:18725641.

Peterson, B., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18**(1): 293–320. doi:10.1146/annurev.es.18.110187.001453.

R Development Core Team. 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org.

Reasoner, M.A., and Hickman, M. 1989. Late Quaternary environmental change in the Lake O'Hara region, Yoho National Park, British Columbia. Palaeogeogr. Palaeoclimatol. Palaeoecol. **72**: 291–316. doi:10.1016/0031-0182(89)90149-1.

Rühland, K., Paterson, A.M., and Smol, J.P. 2008. Hemispheric-scale patterns of climate-related shifts in planktonic diatoms from North American and European lakes. Glob. Change Biol. **14**: 1–15.

Ryves, D., Juggins, S., Fritz, S., and Battarbee, R. 2001. Experimental diatom dissolution and the quantification of microfossil preservation in sediments. Palaeogeogr. Palaeoclimatol. Paleoecol. **172**(1–2): 99–113. doi:10.1016/S0031-0182(01)00273-5.

Saros, J.E., Rose, K.C., Clow, D.W., Stephens, V.C., Nurse, A.B., Arnett, H.A., Stone, J.R., Williamson, C.E., and Wolfe, A.P. 2010. Melting alpine glaciers enrich high-elevation lakes with reactive nitrogen. Environ. Sci. Technol. **44**(13): 4891–4896. doi:10.1021/es100147j. PMID:20527763.

Saros, J.E., Clow, D.W., Blett, T., and Wolfe, A.P. 2011. Critical nitrogen deposition loads in high-elevation lakes of the western US inferred from paleolimnological records. Water Air Soil Pollut. **216**(1–4): 193–202. doi:10.1007/s11270-010-0526-6.

Savard, M.M., Bégin, C., Smirnoff, A., Marion, J., and Rioux-Paquette, E. 2009. Tree-ring nitrogen isotopes reflect anthropogenic NO$_x$ emissions and climatic effects. Environ. Sci. Technol. **43**(3): 604–609. doi:10.1021/es802437k. PMID:19244990.

Schindler, D.W., and Donahue, W.F. 2006. An impending water crisis in Canada's western prairie provinces. Proc. Natl. Acad. Sci. U.S.A. **103**(19): 7210–7216. doi:10.1073/pnas.0601568103. PMID:16606829.

Schindler, D.W., Dillon, P.J., and Schreier, H. 2006. A review of anthropogenic sources of nitrogen and their effects on Canadian aquatic ecosystems. Biogeochemistry, **79**(1–2): 25–44. doi:10.1007/s10533-006-9001-2.

Smol, J.P. 2010. The power of the past: using sediments to track the effects of multiple stressors on lake ecosystems. Freshw. Biol. **55**: 43–59. doi:10.1111/j.1365-2427.2009.02373.x.

Smol, J.P., Birks, H.J.B., and Last, W.M. (*Editors*). 2001. Tracking environmental change using lake sediments, volume 3: terrestrial, algal, and siliceous indicators. Kluwer Academic Publishers, Dortrecht, the Netherlands.

Teranes, J.L., and Bernasconi, S.M. 2000. The record of nitrate utilization and productivity limitation provided by δ^{15}N values in lake organic matter — a study of sediment trap and core sediments from Baldeggersee, Switzerland. Limnol. Oceanogr. **45**(4): 801–813. doi:10.4319/lo.2000.45.4.0801.

Vinebrooke, R., Dixit, S., Graham, M., Gunn, J., Chen, Y.-W., and Belzile, N. 2002. Whole-lake algal responses to a century of acidic industrial deposition on the Canadian Shield. Can. J. Fish. Aquat. Sci. **59**(3): 483–493. doi:10.1139/f02-025.

Vinebrooke, R.D., Cottingham, K.L., Norberg, J., Scheffer, M., Dodson, S.I., Maberly, S.C., and Sommer, U. 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos, **104**: 451–457. doi:10.1111/j.0030-1299.2004.13255.x.

Vinebrooke, R.D., Thompson, P.L., Hobbs, W.O., Luckman, B.H., Graham, M.D., and Wolfe, A.P. 2010. Glacially mediated impacts of climate warming on alpine lakes of the Canadian Rocky Mountains. Verh. Internat. Verein. Limnol. **30**: 1449–1452.

Watson, E., and Luckman, B.H. 2004. Tree-ring-based mass-balance estimates for the past 300 years at Peyto Glacier, Alberta, Canada. Quat. Res. **62**(1): 9–18. doi:10.1016/j.yqres.2004.04.007.

Williamson, C.E., Saros, J.E., and Schindler, D.E. 2009. Sentinels of change. Science, **323**(5916): 887–888. doi:10.1126/science.1169443. PMID:19213905.

Winder, M., Reuter, J.E., and Schladow, S.G. 2009. Lake warming favours small-sized planktonic diatom species. Proc. Biol. Sci. **276**(1656): 427–435. doi:10.1098/rspb.2008.1200. PMID:18812287.

Wolfe, A.P. 1997. On diatom concentrations in lake sediments: results from an inter-laboratory comparison and other tests performed on a uniform sample. J. Paleolimnol. **18**(3): 261–268. doi:10.1023/A:1007937300347.

Wolfe, A.P., Van Gorp, A.C., and Baron, J.S. 2003. Recent ecological and biogeochemical changes in alpine lakes of Rocky Mountain National Park (Colorado, USA): a response to anthropogenic nitrogen deposition. Geobiology, **1**(2): 153–168. doi:10.1046/j.1472-4669.2003.00012.x.

Wunsam, S., Schmidt, R., and Klee, R. 1995. *Cyclotella*-taxa (Bacillariophyceae) in lakes of the Alpine region and their relationship to environmental variables. Aquat. Sci. **57**(4): 360–386. doi:10.1007/BF00878399.

Salmon carcasses alter leaf litter species diversity effects on in-stream decomposition

Welles D. Bretherton, John S. Kominoski, Dylan G. Fischer, and Carri J. LeRoy

Abstract: Marine-derived nutrients from salmon carcasses and leaf litter inputs from riparian vegetation may interactively support stream biodiversity and ecosystem functioning through enhanced resource heterogeneity. Using a full-factorial design of single- and mixed-species litters, we tested for influences of salmon carcasses on in-stream litter decomposition. Overall, nonadditive (synergistic and antagonistic) effects on decomposition were detected for litter species mixtures, and these effects were explained by litter species composition, but not species richness. In middle to late stages of decay, mixtures of labile (high-quality) litters showed faster than expected mass loss, and recalcitrant (low-quality) litter mixtures showed slower than expected mass loss. The presence or absence of each litter species differentially affected decomposition, but these patterns were stronger when salmon carcasses were available. Across all treatments, the influence of salmon carcasses on decomposition was most pronounced in mid-stages of litter decay, where deceleration of decomposition was likely caused by macroinvertebrates feeding on salmon carcasses and less on litter. Combined, these data demonstrate that salmon carcass inputs to streams can enhance detrital heterogeneity, alter interactions among species in litter mixtures, and influence ecosystem functioning (i.e., decomposition).

Résumé : Les nutriments d'origine marine provenant des carcasses de saumons et les apports de litières de feuilles mortes à partir de la végétation riveraine peuvent de façon interactive supporter la biodiversité des cours d'eau et le fonctionnement de l'écosystème en augmentant l'hétérogénéité des ressources. À l'aide d'un plan factoriel complet de litières d'une seule espèce et d'espèces mixtes, nous avons vérifié les effets des carcasses de saumons sur la décomposition des litières dans un cours d'eau. Globalement, on peut détecter des effets (synergiques et antagonistes) non additifs sur la décomposition des litières d'espèces mixtes et ces effets s'expliquent par la composition en espèces de la litière, mais non par la richesse en espèces. Aux étapes moyenne et finale de la décomposition, les mélanges de litière labile (haute qualité) subissent une perte de masse plus rapide que prévu et les mélanges de litière récalcitrante (basse qualité) une perte de masse plus lente que prévu. La présence et l'absence de chaque espèce de litière affectent la décomposition de manière différente, mais ces patrons sont plus marqués lorsque des carcasses de saumons sont présentes. Sur l'ensemble des traitements, l'influence des carcasses de saumons sur la décomposition est plus prononcée aux étapes intermédiaires de la décomposition de la litière, au moment où la décélération de la décomposition s'explique vraisemblablement par l'alimentation des invertébrés plus sur les carcasses de saumons et moins sur la litière. Ces données réunies démontrent que les apports des carcasses de saumons dans les cours d'eau peuvent augmenter l'hétérogénéité du détritus, modifier les interactions des espèces dans les mélanges de litières et affecter le fonctionnement de l'écosystème (c.-à-d. la décomposition).

[Traduit par la Rédaction]

Introduction

Environmental changes are altering the biodiversity of ecosystems worldwide. The functional implications of species losses and the mechanisms of biodiversity effects are largely uncertain (Hooper et al. 2005), yet consistent empirical evidence suggests that nonadditive diversity effects (deviation from the average of individual species) are explained by niche partitioning (Loreau and Hector 2001), niche complementarity (Cardinale et al. 2006), and the increased probability of having species with functionally important traits when species richness increases (e.g., the selection effect; Huston 1997). In autotrophic ecosystems, the species richness of plants has a consistent, positive relationship with aboveground biomass (Cardinale et al. 2007), yet there is no clear relationship between litter species richness and decomposition in heterotrophic terrestrial or aquatic ecosystems (Srivastava et al. 2009). A recent meta-analysis shows that in stream ecosystems, nonadditive effects of litter diversity on decomposition are influenced by the functional dissimilarity of species as well as environmental conditions (Lecerf et al. 2011). Decomposition of diverse litter mixtures is likely influenced by the presence of interacting nutrient sources, but few studies have addressed these interactions (but see Rosemond et al. 2010).

Received 29 December 2010. Accepted 17 May 2011. Published at www.nrcresearchpress.com/cjfas on 23 August 2011.
J2011-0185

W.D. Bretherton, D.G. Fischer, and C.J. LeRoy. The Evergreen State College, 2700 Evergreen Parkway NW, Lab II, 3261, Olympia, WA 98505, USA.
J.S. Kominoski.* Department of Forest Sciences, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada.

Corresponding author: Carri J. LeRoy (e-mail: leroyc@evergreen.edu).

*Present address: Odum School of Ecology, University of Georgia, Athens, GA 30602, USA.

Can. J. Fish. Aquat. Sci. **68**: 1495–1506 (2011) doi:10.1139/F2011-082 **Published by NRC Research Press**

Two important resource subsidies to streams in many locations across the Northern Hemisphere (USA, Canada, Europe, Russia, Japan) are autumnal leaf litter fall and anadromous fish carcasses. In forested headwater streams, the primary energy input is terrestrial leaf fall (Petersen and Cummins 1974; Graça 2001). Additionally, in late fall and early winter, anadromous fish migrate up streams to spawn and subsequently die during the period in which leaf litter is decomposing rapidly (Gende et al. 2002). Four previous studies have examined interactions between salmon carcasses and decomposing leaves (Zhang et al. 2003; Yanai and Kochi 2005; Claeson et al. 2006), but none have addressed the effects of carcasses on the decomposition of leaf litter mixtures composed of several species. These previous studies reported variable effects of salmon-derived nutrients on litter decomposition. In general, salmon carcass presence accelerated litter decomposition (Yanai and Kochi 2005; Claeson et al. 2006, Kohler et al. 2008); however, declines in litter decomposition in the presence of salmon carcasses in one study suggest that shredding invertebrates were drawn to the carcass and away from lower quality litter (Zhang et al. 2003).

Headwater streams in the Pacific Northwest (PNW) USA and Canada can be heavily influenced by salmon carcass influx (Cederholm et al. 1999; Helfield and Naiman 2001; Gende et al. 2002) because of alterations to nutrient budgets and shifts in macroinvertebrate community structure. In general, PNW headwater streams are characterized by low nutrient concentrations owing to underlying parent material, so salmon carcasses provide important energy and nutrient subsidies (Polis et al. 1997). The presence of carcasses in freshwater habitats has been shown to change nutrient dynamics and productivity of algal biofilms (Fisher Wold and Hershey 1999; Johnston et al. 2004; Payne and Moore 2006), as well as increase energy and nutrients for stream food webs (Hocking and Reimchen 2009; Heintz et al. 2010). One study reported that up to 58% of the nitrogen used by macroinvertebrates was from salmon carcasses (Winder et al. 2005). Carcasses in streams have also been shown to increase shredder growth rates (Chaloner and Wipfli 2002; Minakawa et al. 2002) and macroinvertebrate densities (Wipfli et al. 1998; Verspoor et al. 2011) and alter both the trophic structure of stream invertebrate communities (Lessard and Merritt 2006) and the export of insects to adjacent terrestrial forests (Moore and Schindler 2010). Live salmon can have contrasting effects on stream processes and communities through bioturbation (Moore 2006; Honea and Gara 2009; Tiegs et al. 2009) and the physical abrasion of leaf litter (Roberts 2007).

The inclusion of salmon carcasses in litter decomposition experiments could serve as a model for how interacting resources may exaggerate or suppress biodiversity effects on ecosystem function. One recent study in the southeastern USA (Rosemond et al. 2010) compared in-stream decomposition of litter mixtures in the presence or absence of dissolved nutrient additions. Rosemond et al. (2010) found that litter mixing effects on decomposition were suppressed under elevated levels of dissolved inorganic nitrogen and soluble reactive phosphorus. As salmon carcasses can substantially enrich stream water ammonium concentrations (Claeson et al. 2006), we investigated the influence of nutrient-rich salmon carcasses on the relative importance of litter diversity on decomposition. We manipulated salmon carcasses, which occur naturally in many streams, to assess how the presence of carcasses may differentially alter single- and mixed-species litter decomposition and associated macroinvertebrate assemblages. We compared decomposition rates of single- and mixed-species litters from three native riparian tree species using a fully factorial suite of all possible two- and three-species combinations both in the absence and presence of salmon carcasses. We hypothesized that (*i*) both litter species richness and composition in mixtures would influence leaf litter decomposition, based on the inclusion of both labile and recalcitrant litters and increasing resource heterogeneity as richness increases, (*ii*) mixtures of both high- and low-quality litter would decompose nonadditively, but in different directions (synergistically for high-quality litter and antagonistically for low-quality litter), (*iii*) salmon carcass presence would reduce litter diversity effects on decomposition similar to a recent nutrient addition study (Rosemond et al. 2010), and (*iv*) macroinvertebrates would be drawn away from leaf litter and onto carcasses, leading to shifts in the macroinvertebrate community and a deceleration of decomposition when salmon carcasses are present (similar to Zhang et al. 2003).

Materials and methods

Site description

This study was conducted in McLane Creek, located in South Puget Sound, southwest of Olympia, Washington, USA (46°59′57.11″N, 123°01′42.90″W, 175 m elevation). McLane Creek is 23 km in length and contained within a 2980 ha basin. Our study occurred in a 150 m, second-order section of McLane Creek, approximately 3.5 km from the mouth at the Puget Sound. The riparian zone is typical of PNW streams in western Washington and is dominated by red alder (*Alnus rubra*), bigleaf maple (*Acer macrophyllum*), western redcedar (*Thuja plicata*), Douglas-fir (*Pseudotsuga menziesii*), with occasional black cottonwood (*Populus balsamifera* ssp. *trichocarpa*). We chose to focus this study on chum salmon (*Oncorhynchus keta*) because it is the most abundant salmon species in Washington and reliable native and wild run returns in the fall to McLane Creek, where populations have been steadily increasing since 1986 (Washington Department of Fish and Wildlife 2002) and reached over 10 000 in 2008. We chose a small stream with a current salmon run because we were interested in testing the interaction between salmon carcasses and leaf litter mixtures in a stream that naturally receives both types of allochthonous input (leaves and carcasses). This experimental design ensured that stream organisms (microorganisms, invertebrates) were acclimatized to carcass inputs. Although we may have been better able to control presence or absence of salmon carcasses in a non-salmon-bearing stream, we feel the novelty of introducing salmon carcasses to a non-salmon stream could have altered potential outcomes and been logistically difficult (because of fish parasitism and disease transfer). We were only able to install this experiment in one stream, and this limitation should be taken into consideration while interpreting our results. Based on an earlier study that manipulated large quantities of salmon carcasses and showed the strongest nutrient effects within 50 m of massive fish enclosures (containing 58–104 kg of salmon carcasses; Claeson et al. 2006), we designed our study using single carcass enclosures and assumed their influence would be more limited in the downstream direction. We still attempted to spatially separate salmon carcass and no-salmon blocks by at least 10 m to avoid confounding downstream effects.

Leaf decomposition

Senescent leaves from red alder, bigleaf maple, and black cottonwood trees were collected from multiple individuals of each species in a 70-year-old, secondary-growth forest on The Evergreen State College campus forest reserve (8 km north of McLane Creek) just after autumnal abscission using fine mesh nets. Approximately 2 g of air-dried leaves (individual species in mixtures were represented in equal mass proportions) were placed in plastic litter bags (6.4 mm mesh). Previous studies (LeRoy and Marks 2006; Kominoski et al. 2007) have successfully used a similar size mesh, as it allows most shredders access to the leaves, but retains small particles within the bag.

This study consisted of seven leaf litter bag treatments: three species in isolation (alder, cottonwood, maple), three two-species mixtures (1 g of each species placed in litter bags together: alder and cottonwood (A+C), alder and maple (A+M), and cottonwood and maple (C+M)), and one three-species mixture (approximately 0.66 g of each species (A+C +M)). Based on a previous review of phytochemistry and decomposition (Webster and Benfield 1986), we hypothesized alder would decompose fastest, followed by cottonwood, then maple. On 5 December 2006, 210 litter bags (7 litter treatments × 2 salmon carcass treatments × 3 harvest dates × 5 replicates; $n = 5$) were attached to lengths of rebar with cable ties. One replicate litter bag from each litter treatment was placed on each rebar making a total of 30 blocks (15 salmon carcass and 15 no-salmon). Recently deceased (spawned out) chum salmon at roughly the same stage of decomposition (eyes intact) were collected directly from McLane Creek and placed in folded wire enclosures attached directly upstream of the litter bags. No-salmon controls were created using plastic sand bags placed in the same type of folded wire enclosure directly upstream of the litter bags to similarly reduce flow. These salmon carcass and no-salmon blocks were placed on the stream bed and tied to branches and trees along the bank for anchoring. Blocks were placed randomly in similar reduced-flow pools to minimize variation in mechanical breakdown among blocks. Three whole blocks were lost because of high flows, so we were unable to use a blocking variable in our statistical analysis, which should be taken into account when interpreting our results. Owing to the presence of low numbers of salmon carcasses in the stream (unassociated with our salmon carcass treatment), weekly visits to the site were made to remove stray carcasses from near all blocks.

Litter bags were collected after 7, 35, and 78 days of incubation. On each harvest date, litter bags were placed into plastic bags and transported to the lab. Samples were stored at 4 °C and processed within 24–72 h. Litter was carefully rinsed to remove sediment and invertebrates, placed into labeled paper bags and into an oven at 70 °C for 72 h. Invertebrates were then sieved through a 0.25 mm mesh net and preserved in 70% ethanol for later identification. Oven-dried leaf material was ground using a Wiley Mill (Thomas Scientific, Swedesboro, New Jersey, USA) and a subsample (approximately 0.25 g) was combusted in a muffle furnace (Box Furnace, Lindberg/Blue M, Asheville, North Carolina, USA) at 500 °C for 1 h to determine ash-free dry mass (AFDM).

Macroinvertebrate assemblages

Macroinvertebrates from litter bags on harvest dates 1 and 2 were sorted and identified to the lowest possible taxonomic resolution (genus or family in most cases) using a variety of dichotomous keys (Merritt and Cummins 1996; Wiggins 1996; Thorp and Covich 2001) and a dissecting microscope. Examples of each taxon identified were placed in a reference collection in the Field Ecology Lab at The Evergreen State College (Olympia, Washington, USA).

Statistical analysis

Effects on litter decomposition were analyzed based on mass loss on each sampling day as well as analyzing estimated decomposition rates across the study period. First, mass loss from single-species litter bags, in the presence and absence of salmon carcasses, was compared on each sampling day using analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) in SAS version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). Data met the assumptions of normality and equality of variance or were ln-transformed. To test for additive and nonadditive effects of litter species diversity (richness and composition) and salmon carcasses on litter AFDM remaining, we used a full-factorial analysis of covariance (ANCOVA) using PROC GLM in SAS. Additive effects were tested using litter species presence or absence and salmon carcass presence or absence (main effects). Nonadditive effects were measured as significant deviation (synergistic or antagonistic) from the weighted average of mass loss values for the individual component species (with and without salmon carcasses) decomposing in isolation. Significant effects of litter species richness are nonadditive (emergent species interactions), whereas significant effects of litter species composition (single and mixed species assemblages) can be either additive or nonadditive. Time was treated as a continuous variable in the model, and each term (time, litter species presence or absence and salmon carcass presence or absence (additivity), and a diversity term (nonadditivity; richness and composition)) was added sequentially to the model. The diversity term was composed of mixed-species richness (one-, two-, or three-species litter mixtures) and mixed-species composition (A+C, A+M, C+M, A+C +M). Interactions of each term with time were also added to the model. Model effects were fixed, and type I sums of squares were used to determine significant treatment effects (Kominoski et al. 2007; Kominoski and Pringle 2009). The order of main effects terms was altered and models were rerun to assure that there were no differences among terms associated with their sequence in the model. Significant main effects indicated additivity (no significant deviation from expected), litter species, or salmon carcass presence or absence effects. Significant nonadditivity (synergism or antagonism) was indicated by effects of litter species richness, litter species composition, or both. If nonadditivity was measured, we re-ran the full-factorial analysis to test for effects of litter species richness. If litter species richness effects were not significant, we determined nonadditivity to be attributed to litter species composition. The influence of the presence or ab-

Table 1. Analysis of variance (ANOVA) results for a general linear model describing the effects of incubation time (Time), leaf species presence or absence, salmon carcass presence or absence (Salmon), litter species richness (Richness), and litter species composition (Composition) within leaf litter bags, as well as interactions among these factors on leaf litter mass loss.

	Type I				
Source	*df*	SS	MS	*F*	*p*
Time	**2**	**5.92**	**2.96**	**167.31**	**<0.0001**
A	**1**	**0.11**	**0.11**	**6.01**	**0.0153**
C	**1**	**0.22**	**0.22**	**12.35**	**0.0006**
M	**1**	**0.47**	**0.47**	**26.57**	**<0.0001**
Salmon	1	0.06	0.06	3.19	0.0761
Richness	1	0.04	0.04	2.34	0.1283
Composition	**2**	**0.13**	**0.07**	**3.76**	**0.0254**
A × Salmon	1	0.03	0.03	1.53	0.2183
C × Salmon	1	0.01	0.01	0.10	0.7484
M × Salmon	1	0.01	0.01	0.40	0.5279
Salmon × Richness	1	0.003	0.003	0.20	0.6524
Salmon × Composition	2	0.04	0.02	1.00	0.3688
Time × A	2	0.07	0.03	1.93	0.1490
Time × C	2	0.02	0.01	0.64	0.5309
Time × M	2	0.02	0.01	0.58	0.5591
Time × Salmon	**2**	**0.14**	**0.07**	**3.99**	**0.0204**
Time × Richness	2	0.02	0.01	0.60	0.5491
Time × Composition	4	0.13	0.03	1.89	0.1141
Time × A × Salmon	2	0.01	0.004	0.22	0.8024
Time × C × Salmon	2	0.01	0.004	0.23	0.7955
Time × M × Salmon	2	0.01	0.01	0.34	0.7121
Time × Salmon × Richness	2	0.05	0.03	1.48	0.2315
Time × Salmon × Composition	4	0.04	0.01	0.60	0.6627

Note: This model uses fixed effects and type I sums of squares. Litter species diversity effects are separated into the effects of species richness and species composition. Note that a type I (sequential) *F* test is used here, so the order in which variables are placed into the model can alter subsequent *F* ratios. Order was randomized and no differences were found among effect sizes that would alter interpretation. Significant effects are denoted in bold. A, red alder (*Alnus rubra*); C, black cottonwood (*Populus trichocarpa*); M, bigleaf maple (*Acer macrophyllum*).

sence of each litter species in litterbags on mass loss was determined using two-tailed *t* tests.

Second, we also analyzed decomposition rates across the study period. Decomposition rate constants (k) were determined for each leaf litter treatment with and without salmon carcasses by regressing the natural log of AFDM against day (Jenny et al. 1949). All exponential regressions were significant at $\alpha < 0.05$. Analyses of k were conducted separately using PROC MIXED in SAS (Swan and Palmer 2004). To determine the percentage of the variation in mass loss explained by litter treatments, salmon carcass treatments, and litter × salmon interactions at each harvest date, samples were reclassified as random effects and simple two-way ANOVAs were performed.

Macroinvertebrate data from litter bags were analyzed using both basic and comprehensive community analysis methods. First, invertebrate abundance, taxa richness, taxa evenness, and taxa diversity indices (Shannon's H' and Simpson's D) were compared among treatments using two-way ANOVAs in JMP (7.0.2, SAS Institute Inc., Cary, North Carolina). Second, community analysis methods including nonmetric multidimensional scaling ordination and permutative multivariate ANOVA (PERMANOVA; Anderson 2001) were performed in PC-ORD (4.34, MjM Software Design, http://home.centurytel.net/~mjm/). Two-way PERMANOVAs were used to determine significant effects of leaf treatments, salmon carcass treatments, and their interaction on macroinvertebrate assemblages. Indicator species analysis was used to determine species-specific associations with litter or salmon carcass treatments (Dufrene and Legendre 1997). For all analyses, $\alpha = 0.05$ was used to determine statistical significance.

Results

Litter mass loss

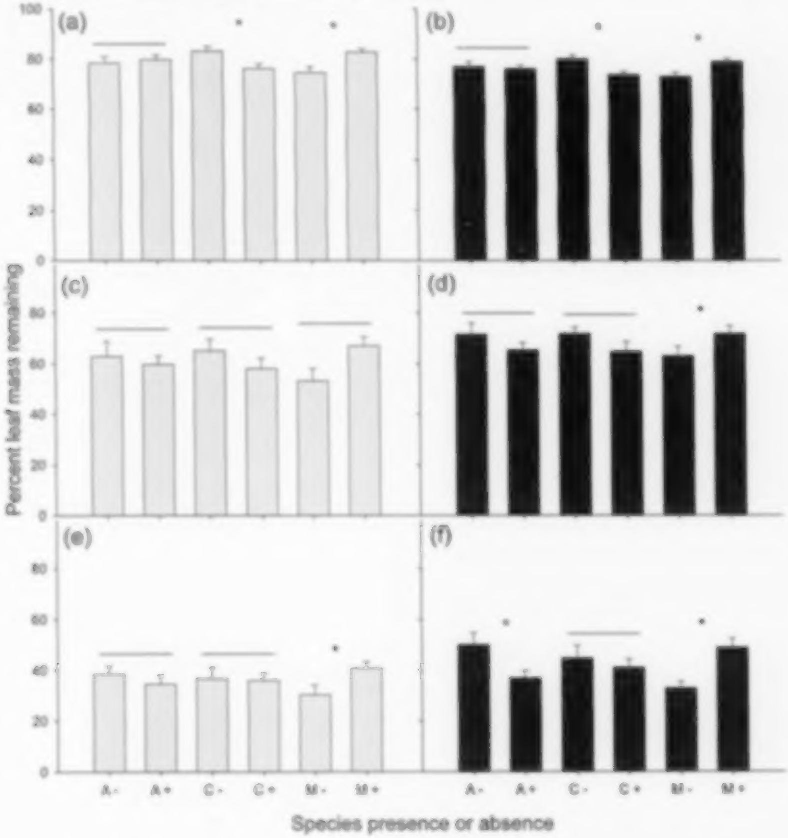
Overall model

Leaf litter mass loss was significantly influenced by time in the stream, the presence or absence of each species (alder, maple, and cottonwood), and litter species composition, but not salmon carcass presence or litter species richness (Table 1). A species composition effect denotes significant nonadditive patterns of decomposition for various species mixtures. There were no significant effects of any interactions, except for a time-dependent effect of salmon carcass presence or absence on in-stream mass loss (Table 1).

Species presence or absence

Looking more closely at patterns found at each harvest date, we found several time-dependent patterns in mass loss based on the presence or absence of each species (Fig. 1).

Fig. 1. The influence of the presence or absence of each litter species on percent mass remaining at each harvest date in both no-salmon control (gray bars) and salmon carcass manipulation (black bars) treatments for three harvests: (*a* and *b*) harvest 1 (7 days), (*c* and *d*) harvest 2 (35 days), and (*e* and *f*) harvest 3 (78 days). Litter species are shown on the *x* axis: A, red alder (*Alnus rubra*); C, black cottonwood (*Populus trichocarpa*); and M, bigleaf maple (*Acer macrophyllum*), as well as the presence (+) or absence (–) of each species in a given litter bag. Mass remaining values were averaged across all litter bags including or excluding each species at each harvest date and salmon treatment. Significant differences between the presence and absence of a given species are denoted with an asterisk (*) between each pair of bars. Nonsignificant differences are denoted by a horizontal line over each pair of bars.



On harvest day 7, in both the absence and the presence of salmon carcasses there was significantly more mass remaining in litter bags without *P. trichocarpa* (no-salmon: $t_{[27]} = -3.22$, $p = 0.0032$; salmon: $t_{[30]} = -2.57$, $p = 0.0151$) and with *A. macrophyllum* (no-salmon: $t_{[27]} = 3.02$, $p = 0.0052$; salmon: $t_{[26]} = 2.91$, $p = 0.0071$). On harvest day 35, in the absence of salmon carcasses there are no significant effects of litter species presence or absence for any of the species ($p > 0.05$), but in the presence of salmon carcasses, the presence of *A. macrophyllum* litter significantly slowed decomposition ($t_{[27]} = 2.26$, $p = 0.0317$). On harvest day 78, in the absence of salmon carcasses, only the presence of *A. macrophyllum* reduced decomposition ($t_{[24]} = -2.32$, $p = 0.0289$), but in the presence of salmon carcasses both litter bags without alder ($t_{[19]} = -2.41$, $p = 0.0260$) and litter bags with *A. macrophyllum* ($t_{[25]} = 3.53$, $p = 0.0016$) decomposed significantly slower (Fig. 1).

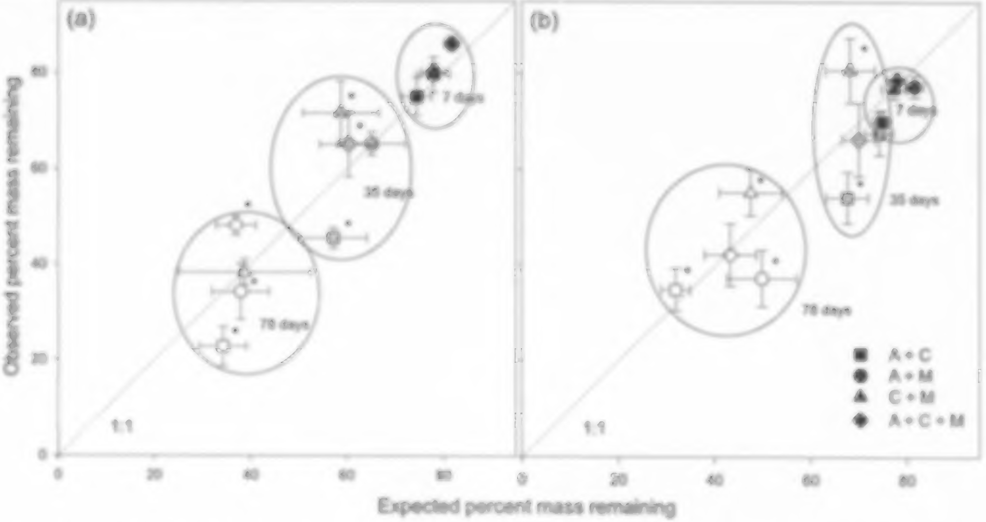
Nonadditive effects

Litter mass remaining at each harvest date was not predictable based on additive constituent species combinations and was therefore nonadditive (both synergistic and antagonistic) for most treatments. These patterns are visualized using observed versus expected graphs of mass remaining, where points that place above the 1:1 line decompose slower than expected (Fig. 2). Average mass remaining among mixed-species litter treatments showed additive decomposition across all treatments at harvest day 7, but both synergistic (faster than expected) and antagonistic (slower than expected) mass loss on harvest days 35 and 78 and in both salmon carcass and no-salmon control treatments. Although there are some minor discrepancies among salmon carcass treatments, in general the A+C and A+M mixtures show more synergistic mass loss, and the C+M and A+C+M mixtures show more antagonistic mass loss (Fig. 2).

Salmon carcass presence or absence

Salmon carcass presence generally decelerated litter decomposition for all litter treatments, but the time-dependent effects of salmon carcass treatments show a stronger influ-

Fig. 2. Observed percent mass remaining for all litter mixture treatments plotted as a function of the expected percent mass remaining based on average values of mass remaining for each species in isolation for (*a*) all no-salmon litter bags and (*b*) all salmon carcass treatment litterbags. Leaf litter treatments included the following mixtures: alder + cottonwood (A+C; ■), alder + maple (A+M; ●), cottonwood + maple (C+M; ▲) and alder + cottonwood + maple (A+C+M; ◆). Black symbols represent litterbags collected after 7 days, dark gray symbols were collected after 35 days, and open symbols were collected after 78 days. Diagonal line represents 1:1 equilibrium, and asterisks (*) denote nonadditivity. Points that fall above the 1:1 line indicate significantly more mass remaining than predicted (antagonism). Ellipses contain all means from each harvest date.



ence of carcasses in the middle and late stages of decay (Fig. 3). When we plot the no-salmon control values of average mass remaining (as expected rates given no salmon effect) against the values for the salmon carcass treatments, we see that most litter treatments (except the A+C+M mixture) place above the 1:1 line (Fig. 3), showing slower than expected mass loss.

Litter decomposition rates

Decomposition rates (k) integrate mass loss data across all time intervals. Our data show significant differences in k among single-species treatments (A vs. C vs. M) with salmon carcasses present, but no differences when salmon carcasses were absent (Fig. 4). Specifically, *A. macrophyllum* litter decomposed significantly slower than *A. rubra* or *P. trichocarpa* when salmon carcasses were present (Fig. 4*b*; M vs. C: $F_{[1,126]} = 14.11$, $p = 0.0003$; M vs. A: $F_{[1,126]} = 8.30$, $p = 0.0047$). However, when salmon carcasses were absent, there was no significant variation among the single species litter treatments (Fig. 4*a*; $p > 0.05$). In contrast, we show statistically more variation among k for the mixed-species treatments when salmon carcasses were absent than present. Although the A+C mixture decomposed significantly faster than the C+M mixture when carcasses were present (Fig. 4*b*; $F_{[1,126]} = 4.45$, $p = 0.0369$), the A+C mixture decomposed faster than all other mixtures when carcasses were absent (Fig. 4*a*; $p < 0.05$).

Percent variation explained

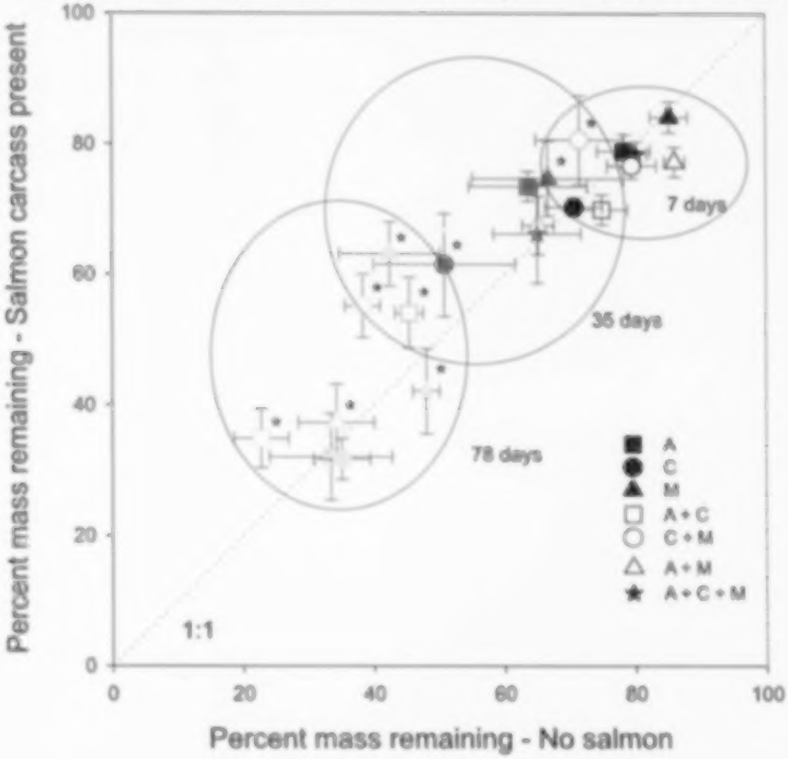
Although there are some influences of salmon carcasses on leaf litter decomposition rates, most of the variation in the model was explained by litter treatment, not salmon carcass treatment. Differences among litter treatments explained the majority of the variation in decomposition rates (Fig. 5). Initially 31% of the variation in k was explained by differences among litter treatments. This effect was slightly diminished, though still significant, throughout the decomposition process, resulting in 19.7% explanation by day 78. The presence of salmon carcasses only explained ~5% of the variation in k throughout the entire process. Interestingly, the litter by salmon carcass interaction, although relatively insignificant throughout the study, showed a slight increase (0% to 10%) in percent variation explained through time, in contrast with the decrease in the variation explained by the litter treatments (Fig. 5).

Macroinvertebrates

Community structure

We found a significant effect of the salmon carcass treatment on whole community macroinvertebrate composition through PERMANOVA for both harvest 1 ($F_{[1,56]} = 2.16$, $p = 0.0236$) and harvest 2 ($F_{[1,56]} = 3.36$, $p = 0.0008$), but insignificant effects of leaf treatment or leaf × salmon carcass interactions ($p > 0.05$). Comparing observed macroinvertebrate communities in litter mixtures with those expected based on communities in individual species litter bags showed additive community structure for all mixtures ($p > 0.05$). However, when we restricted the macroinvertebrate community to only shredding invertebrates, we found a significant effect of leaf treatment at harvest 1 ($F_{[6,56]} = 2.26$, $p = 0.0080$), but insignificant effects of the salmon carcass treatment or leaf × salmon carcass interaction ($p > 0.05$). In this case, cottonwood litter hosted a significantly different shredder community than all other leaf treatments except alder.

Fig. 3. Observed percent mass remaining for all leaf litter treatments in the presence of salmon carcasses (*y* axis) plotted as a function of the expected decomposition rates based on the no-salmon controls (*x* axis). Leaf litter treatments included alder (A; ■), cottonwood (C; ●), and maple (M; ▲), and mixed treatments consisted of alder + cottonwood (A+C; □), cottonwood + maple (C+M; ○) alder + maple (A+M; △), and alder + cottonwood + maple (A+C+M; ★). Ellipses contain all means from each harvest date (black symbols and outlines represent litterbags collected after 7 days, dark gray symbols and outlines were collected after 35 days, and light gray symbols and outlines were collected after 78 days). Diagonal line represents 1:1 equilibrium, and asterisks (*) help to illustrate treatments that deviated from expectation.



Community metrics

Using simple community metrics, total shredder abundance was significantly influenced by leaf litter treatment, but not salmon carcass treatment or the leaf × salmon carcass interaction using two-way ANOVA ($F_{[6,56]} = 3.03$, $p = 0.0124$). In this case, cottonwood litter and mixtures including cottonwood litter hosted significantly lower numbers of shredders (Figs. 4*c*, 4*d*). However, using the entire macroinvertebrate community, simple community metrics showed no response to litter treatments, salmon carcass treatments, or their interaction using two-way ANOVA for either harvest 1 or harvest 2 (Table 2). The number of macroinvertebrates (total abundance) in each litter bag was quite variable (ranged from 1 to 71) and represented a diversity of functional feeding guilds and invertebrate families. Although insects colonizing salmon carcasses were not surveyed for this study (those surveys were beyond the scope of this study), observationally on visits to the stream site we saw invertebrates of many types actively feeding on the salmon carcasses (Gastropoda, Trichoptera, Diptera, etc.).

Indicator species analysis

Indicator species analysis highlights species that show fidelity to certain treatments. In this study, three genera significantly indicated for salmon carcass absence (Ephemeroptera: *Ironodes* sp. (indicator value (IV) = 36.2, $p = 0.0112$); Ephemeroptera: *Baetis* sp. (IV = 41.0, $p = 0.0022$); Glossiphoniidae: *Helobdella* sp. (IV = 50.5, $p = 0.0012$)), two genera significantly indicated for the highest litter species richness level (three-species mixture; Plecoptera: *Isoperla* sp. (IV = 21.4, $p = 0.0330$); Glossiphoniidae: *Helobdella* sp. (IV = 70.0, $p = 0.0230$)), and one species significantly indicated for the C+M mixture (Ephemeroptera: *Seratella* sp. (IV = 23.5, $p = 0.0444$)). The ephemeropteran *Baetis* sp. also indicated for the presence of *A. macrophyllum* litter when carcasses were unavailable (IV = 25.0, $p = 0.0490$), and the plecopteran, *Zapada* sp. indicated for the absence of *P. trichocarpa* leaf litter (IV = 54.2, $p = 0.0090$) and the presence of *A. macrophyllum* litter (IV = 51.8, $p = 0.0150$), but only when carcasses were available.

Discussion

Salmon carcass presence and litter species composition, but not species richness, interactively affected leaf litter decomposition. Specifically, we detected more synergistic effects of mixing for high-quality litters and more antagonistic effects of mixing for low-quality litters on decomposition, but we also measured some additive responses to litter mixing.

Fig. 4. Decomposition rates ($k \cdot day^{-1}$) and shredder abundances (total number per litterbag) for each litter species in isolation: red alder (A, *Alnus rubra*), black cottonwood (C, *Populus balsamifera* ssp. *trichocarpa*), and bigleaf maple (M, *Acer macrophyllum*), and mixed treatments consisting of alder + cottonwood (A+C), alder + maple (A+M), cottonwood + maple (C+M) and alder + cottonwood + maple (A+C+M). Panels (*a*) and (*c*) show litterbags placed downstream of a sandbag (no-salmon control block, gray bars), and (*b*) and (*d*) show litter bags placed downstream of a salmon carcass (black bars). Lowercase letters denote significant differences among treatments at $\alpha = 0.05$.

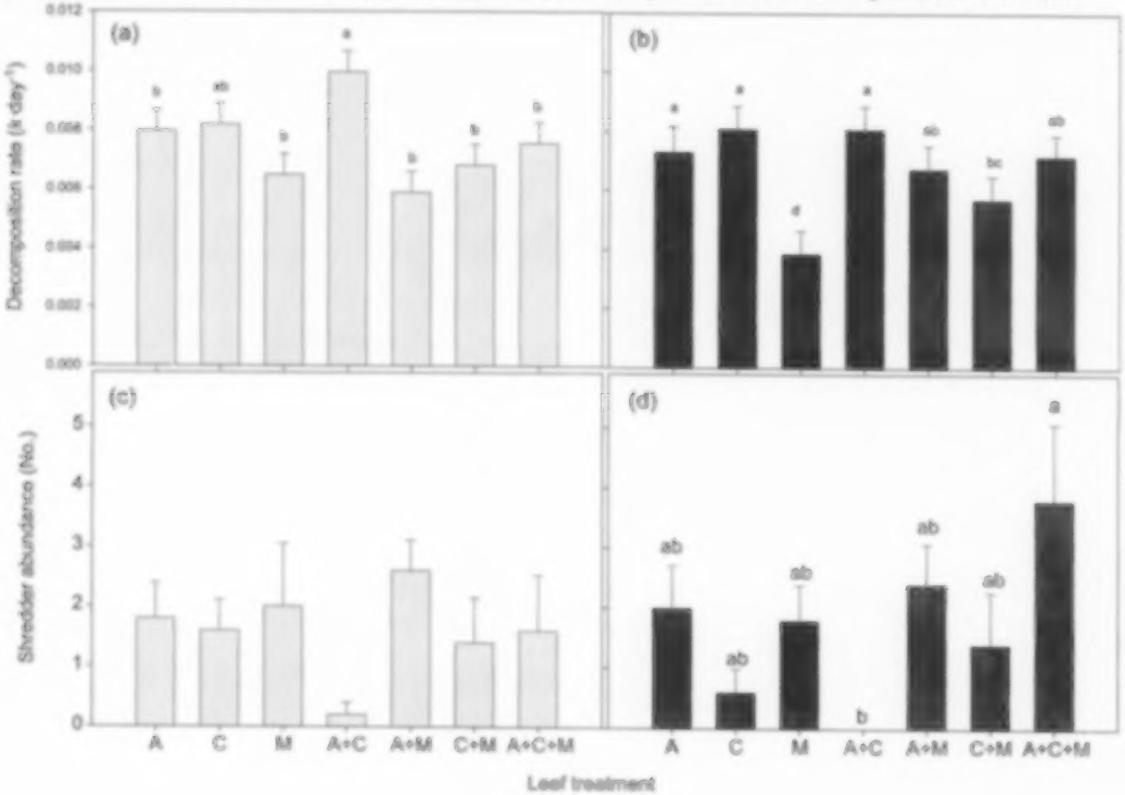


Fig. 5. Percent variance explained by differences among the leaf treatments (●), the presence of salmon carcasses (▼), and the interaction between salmon carcass presence and leaf litter treatments (■). Asterisks (*) denote significant proportions of the variation explained by a given factor ($\alpha = 0.05$).

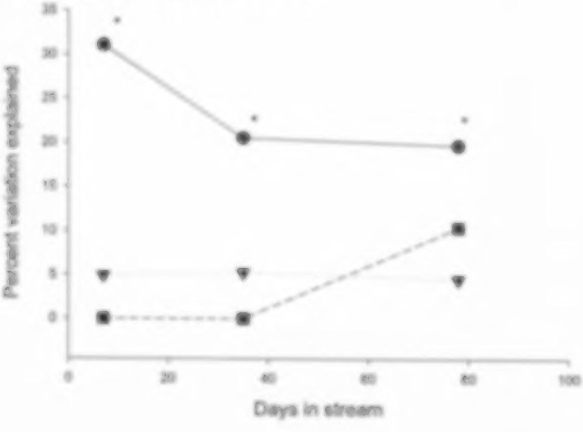


Table 2. Simple macroinvertebrate community metrics did not respond to leaf litter treatments, salmon carcass treatments, or their interaction.

Community metric	df	*F*	*p*
Harvest 1			
Total macroinvertebrate abundance	13, 56	1.12	0.3608
Taxa richness	13, 56	0.80	0.6541
Taxa evenness	13, 56	1.13	0.3521
Shannon's diversity index (*H′*)	13, 56	0.87	0.5872
Simpson's diversity index (*D*)	13, 56	1.00	0.4587
Harvest 2			
Total macroinvertebrate abundance	13, 56	1.13	0.3472
Taxa richness	13, 56	1.04	0.4249
Taxa evenness	13, 56	1.00	0.4632
Shannon's diversity index (*H′*)	13, 56	0.69	0.7593
Simpson's diversity index (*D*)	13, 56	0.73	0.7229

Although salmon carcass presence did not have a strong main effect on decomposition, we found a significant time-dependent effect of salmon carcasses on both decomposition and litter diversity effects, as well as some deceleration of decomposition in the presence of carcasses. Salmon carcass presence significantly influenced macroinvertebrate communities, and shredder communities varied based on leaf litter treatments. These results have implications for management and restoration of both riparian and stream ecosystems in the PNW, which are influenced by tree species diversity and quantities of returning salmon.

Our results demonstrate that resource heterogeneity influences stream ecosystem functioning. Based on previous work, we hypothesized that both species richness and species composition in mixed litter treatments would significantly influence leaf litter decomposition; however, we found evidence for only compositional effects and no support for richness effects. Compositionally, litter bags containing mixtures that included maple litter (the most recalcitrant species) showed significantly slower mass loss than mixtures that did not include maple litter (regardless of salmon carcass presence or absence). Another compositional pattern early in the decomposition process involved faster mass loss for mixtures containing cottonwood litter.

Similarly, we hypothesized that litter mixtures would decompose nonadditively, and this hypothesis was supported by our data showing both synergistic and antagonistic responses of decomposition to litter mixing. Although many treatments showed additive patterns, in general, the most recalcitrant mixture (which included cottonwood and maple; C+M) showed antagonistic responses in mixtures, which could be due to physical protection against fragmentation conferred by the recalcitrant litter. In contrast, the more labile mixtures with alder (A+C, A+M) responded synergistically, possibly resulting from nutrient transfer among species, especially the nitrogen-fixing alder. However, more detailed study would be necessary to determine if these are the mechanisms that are, in fact, acting on these different mixtures. The antagonisms seen for the recalcitrant mixtures are supported by several studies showing reduced mass loss for mixtures containing recalcitrant species (Swan and Palmer 2004; Kominoski et al. 2007). Conversely, the synergisms shown for labile mixtures are supported by another study (which used another relatively labile *Alnus* species; LeRoy and Marks 2006) and a recent review paper (Lecerf et al. 2011). Additionally, the A+C+M mixture appeared to be the least sensitive of all mixtures to nonadditive effects, though it is possible that decomposition rates for each species were cancelled by one another in the three-species mixture. We were unable to separate individual leaf litter species within mixtures, which may have given insight into these patterns. Finally, although salmon carcasses did not completely overwhelm litter diversity effects as hypothesized, the presence of carcasses interacted with litter mixtures, resulting in altered responses for most mixtures.

Environmental context is a critical component explaining diversity effects on ecosystem functioning (Cardinale et al. 2000). We hypothesized that salmon carcasses might overwhelm litter diversity effects. Based on recent research that highlights how litter mixture effects on decomposition may be relative to the presence of other resources, such as dissolved nutrients (Rosemond et al. 2010), we expected to see an interaction between salmon carcasses and litter mixing on decomposition. In our study, salmon carcasses did not override litter diversity effects on decomposition. Rather, salmon presence tended to accentuate differences in decomposition among species and magnify macroinvertebrate responses to litter mixing. Finally, salmon carcasses showed a consistently weaker effect on the decomposition process compared with the litter treatment effects. We did, however, see time-dependent effects on mass remaining that varied with salmon carcass presence or absence. The presence of salmon carcasses generally resulted in slower mass loss across treatments, especially at mid-stages of decay. This is in contrast with accelerated decomposition seen in Claeson et al. (2006) and Rosemond et al. (2010), but is in accordance with slowed decomposition seen in Zhang et al. (2003). In both Claeson et al. (2006) and Rosemond et al. (2010), elevated nutrients were available to detrital communities downstream, which stimulated decomposition rates. In our study, although nutrients were likely elevated directly downstream of single carcass manipulations (data not collected), the carcasses themselves were available as habitat to shredding invertebrates and likely influenced macroinvertebrate feeding preferences as in Zhang et al. (2003).

The strength of species complementarity effects often increases with time (Cardinale et al. 2007; Srivastava et al. 2009; Lecerf et al. 2011), and we showed a trend of increasing nonadditivity in litter mass remaining over time resulting in antagonistic effects of salmon carcasses.

The decomposition patterns shown here could be explained by three potential mechanisms. First, there is little influence of salmon carcasses on litter leaching processes early in the decomposition process (the likely dominant process at day 7). Second, there are increasingly important interactions among salmon carcasses, microorganisms, and macroinvertebrates at mid-stages of decay (35 days) when salmon-derived ammonium levels peak (Claeson et al. 2006). Macroinvertebrate communities differed among salmon carcass treatments, and shredder communities differed among leaf treatments. Several indicator species showed preferences for certain conditions: maple presence, cottonwood absence, salmon carcass presence, high litter species richness. However, patterns of nonadditive macroinvertebrate community dynamics are not as strong as in past studies on litter mixing. Kominoski and Pringle (2009) found both additive and nonadditive patterns for both decomposition and macroinvertebrate colonization. When salmon carcasses are present, it is likely that macroinvertebrates preferentially consume salmon tissue over leaf litter, which may have resulted in the inhibitory effects of salmon carcass presence on *A. macrophyllum* litter decomposition (Zhang et al. 2003). Third, by day 78 leaf materials are reduced to recalcitrant components resistant to decay (regardless of carcass presence), and salmon carcasses have mostly been decomposed, diminishing their effects. Claeson et al. (2006) showed no influence of salmon carcasses on downstream ammonium after 79 days, and it is likely our nutrient pulse had diminished by this time as well. In comparing litter bags in the absence and presence of salmon carcasses, the deviation away from the 1:1 line shows a successive deceleration of decomposition in the presence of carcasses. It is unclear whether this deviation would continue or whether decomposition patterns would return to expectation past 78 days. A more complete study including more frequent harvests and a variety of microbial measurements would provide resolution to this question.

Leaf litter inputs and salmon carcass deposition represent the two most important allochthonous inputs to small streams in the PNW. This study helps us understand the complicated interactions between these two inputs and how detrital organisms may shift feeding among sources on a short temporal scale and at relatively small spatial scales. Because of the widespread loss of riparian forests (Sweeney et al. 2004) and the precipitous declines in salmonid populations (only 6%–7% of historic PNW populations persist; Gresh et al. 2000), riparian restoration practices often include either riparian revegetation or salmon carcass additions (or both). Vast tracts of riparian forest are being replanted with a variety of riparian species (but often at unnatural levels of species or genetic variation) to create forest habitat and restore the sources of woody debris that increase in-stream heterogeneity and habitat for salmon. In addition, in an effort to fertilize low-productivity forests and streams in the PNW, a variety of agencies have initiated "salmon carcass toss" programs to reintroduce salmon-derived nutrients into systems where diminished populations of salmon are no longer fertilizing forests and streams. Although these two restoration practices are common, their interactions are understudied.

Riparian forested and stream ecosystems are linked through subsidies of energy and nutrients, and the spatial, temporal, and compositional dynamics of these resources influence ecosystem functioning in salmonid watersheds (Wipfli and Baxter 2010). Our data highlight five major findings linking litter diversity, salmon carcass inputs, stream ecosystem structure, and functioning. First, litter composition had a stronger effect than richness on the decomposition process, and thus riparian leaf traits may have a stronger influence on in-stream processes than measures of diversity like litter richness. Second, the influence of a keystone fish species (Willson and Halupka 1995) on litter dynamics had a mild decelerating influence on decomposition and altered ecological interactions among plant litter species. Third, although salmon carcass presence may decelerate litter decomposition overall, litter species composition appeared to explain the majority of the variation in litter decomposition rates, but compositional effects were dependent on the presence or absence of salmon carcasses. Thus, salmon carcasses changed the nature of litter composition effects. Fourth, the strength of the salmon carcasses influence was dynamic through time, with the strongest effects shown at mid-stages of decay, likely correlated with higher salmon-derived ammonium concentrations (Claeson et al. 2006). Fifth, the presence of salmon carcasses may have drawn stream macroinvertebrates away from litter, as salmon carcasses are a more labile source of nutrients and energy. However, no reductions in total macroinvertebrate abundance were seen with respect to the presence of salmon carcasses, although whole community differences were shown through PERMANOVA. Our results suggest that plant species mixtures may be at least as (or more) important in determining stream carbon cycling rates than the presence of large numbers of decomposing vertebrate species, and diverse detrital inputs may lead to complex, unexpected synergistic and antagonistic outcomes. Although other studies have demonstrated synergistic and antagonistic effects in plant species mixtures, this is the first study to explicitly integrate the influence of salmon carcasses, as another component of detrital diversity, on stream ecosystem functioning.

Acknowledgements

Thank you to the Evergreen State College Field Ecology Lab: K. Halstead, C. Anthony, and P. Babbin. Assistance was provided by D.G. Fischer and R. Cole's Introduction to Environmental Studies, Fall 2006 undergraduate class. Access to field sites was provided by the Washington Department of Natural Resources. Several anonymous reviewers provided feedback that substantially improved this manuscript.

References

Anderson, M.J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. **26**: 32–46.

Cardinale, B.J., Nelson, K., and Palmer, M.A. 2000. Linking species diversity to the functioning of ecosystems: on the importance of environmental context. Oikos, **91**(1): 175–183. doi:10.1034/j.1600-0706.2000.910117.x.

Cardinale, B.J., Srivastava, D.S., Duffy, J.E., Wright, J.P., Downing, A.L., Sankaran, M., and Jouseau, C. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature, **443**(7114): 989–992. doi:10.1038/nature05202. PMID:17066035.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M., and Weis, J.J. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proc. Natl. Acad. Sci. U.S.A. **104**(46): 18123–18128. doi:10.1073/pnas.0709069104. PMID:17991772.

Cederholm, C.J., Kunze, M.D., Murota, T., and Sibatani, A. 1999. Pacific salmon carcasses: essential contributions of nutrients and energy for aquatic and terrestrial ecosystems. Fisheries, **24**: 6–15.

Chaloner, D.T., and Wipfli, M.S. 2002. Influence of decomposing Pacific salmon carcasses on macroinvertebrate growth and standing stock in southeastern Alaska streams. J. N. Am. Benthol. Soc. **21**(3): 430–442. doi:10.2307/1468480.

Claeson, S.M., Li, J.L., Compton, J.E., and Bisson, P.A. 2006. Response of nutrients, biofilm, and benthic insects to salmon carcass addition. Can. J. Fish. Aquat. Sci. **63**(6): 1230–1241. doi:10.1139/f06-029.

Dufrene, M., and Legendre, P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. **67**: 345–366.

Fisher Wold, A.K., and Hershey, A.E. 1999. Effects of salmon carcass decomposition on biofilm growth and wood decomposition. Can. J. Fish. Aquat. Sci. **56**(5): 767–773. doi:10.1139/f99-030.

Gende, S.M., Edwards, R.T., Willson, M.F., and Wipfli, M.S. 2002. Pacific salmon in aquatic and terrestrial ecosystems. Bioscience, **52**(10): 917–928. doi:10.1641/0006-3568(2002)052[0917:PSIAAT]2.0.CO;2.

Graça, M.A.S. 2001. The role of invertebrates on leaf litter decomposition in streams — a review. Int. Rev. Hydrobiol. **86**(4–5): 383–393. doi:10.1002/1522-2632(200107)86:4/5<383::AID-IROH383>3.0.CO;2-D.

Gresh, T., Lichatowich, J., and Schoonmaker, P. 2000. An estimation of historic and current levels of salmon production in the Northeast Pacific ecosystem: evidence of a nutrient deficit in the freshwater systems of the Pacific Northwest. Fisheries, **25**: 15–21.

Heintz, R.A., Wipfli, M.S., and Hudson, J.P. 2010. Identification of marine-derived lipids in juvenile coho salmon and aquatic insects through fatty acid analysis. Trans. Am. Fish. Soc. **139**(3): 840–854. doi:10.1577/T09-115.1.

Helfield, J.M., and Naiman, R.J. 2001. Effects of salmon-derived nitrogen on riparian forest growth and implications for stream productivity. Ecology, **82**(9): 2403–2409. doi:10.1890/0012-9658(2001)082[2403:EOSDNO]2.0.CO;2.

Hocking, M.D., and Reimchen, T.E. 2009. Salmon species, density and watershed size predict magnitude of marine enrichment in riparian food webs. Oikos, **118**(9): 1307–1318. doi:10.1111/j.1600-0706.2009.17302.x.

Honea, J.M., and Gara, R.I. 2009. Macroinvertebrate community dynamics: strong negative response to salmon red construction and weak response to salmon-derived nutrient uptake. J. N. Am. Benthol. Soc. **28**(1): 207–219. doi:10.1899/08-030.1.

Hooper, D.U., Chapin, F.S., III, Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setl, H., Symstad, A.J., Vandermeer, J., and Wardle, D.A. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol. Monogr. **75**(1): 3–35. doi:10.1890/04-0922.

Huston, M.A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia, **110**(4): 449–460. doi:10.1007/s004420050180.

Jenny, H., Gessel, S.P., and Bingham, F.T. 1949. Comparative study of decomposition rates of organic matter in temperate and tropical regions. Soil Sci. **68**(6): 419–432. doi:10.1097/00010694-194912000-00001.

Johnston, N., MacIsaac, E., Tschaplinski, P., and Hall, K. 2004. Effects of the abundance of spawning sockeye salmon (*Oncorhynchus nerka*) on nutrients and algal biomass in forested streams. Can. J. Fish. Aquat. Sci. **61**(3): 384–403. doi:10.1139/f03-172.

Kohler, A.E., Rugenski, A., and Taki, D. 2008. Stream food web response to a salmon carcass analogue addition in two central Idaho, U.S.A. streams. Freshw. Biol. **53**(3): 446–460. doi:10.1111/j.1365-2427.2007.01909.x.

Kominoski, J.S., and Pringle, C.M. 2009. Resource–consumer diversity: testing the effects of leaf litter species diversity on stream macroinvertebrate communities. Freshw. Biol. **54**(7): 1461–1473. doi:10.1111/j.1365-2427.2009.02196.x.

Kominoski, J.S., Pringle, C.M., Ball, B.A., Bradford, M.A., Coleman, D.C., Hall, D.B., and Hunter, M.D. 2007. Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. Ecology, **88**(5): 1167–1176. doi:10.1890/06-0674. PMID:17536403.

Lecerf, A., Marie, G., Kominoski, J., LeRoy, C., Bernadet, C., and Swan, C.M. 2011. Incubation time, functional litter diversity, and habitat characteristics predict litter-mixing effects on decomposition: A synthesis from streams. Ecology, **92**: 160–169. doi:10.1890/10-0315.1. PMID:21560686.

LeRoy, C.J., and Marks, J.C. 2006. Litter quality, stream characteristics and litter diversity influence decomposition rates and macroinvertebrates. Freshw. Biol. **51**(4): 605–617. doi:10.1111/j.1365-2427.2006.01512.x.

Lessard, J.L., and Merritt, R.W. 2006. Influence of marine-derived nutrients from spawning salmon on aquatic insect communities in southeast Alaskan streams. Oikos, **113**(2): 334–343. doi:10.1111/j.2006.0030-1299.14512.x.

Loreau, M., and Hector, A. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature, **412**(6842): 72–76. doi:10.1038/35083573. PMID:11452308.

Merritt, R.W., and Cummins, K.W. 1996. An introduction to the aquatic insects of North America. 3rd ed. Kendall-Hunt Publishing Co., Dubuque, Iowa, USA.

Minakawa, N., Gara, R.I., and Honea, J.M. 2002. Increased individual growth rate and community biomass of stream insects associated with salmon carcasses. J. N. Am. Benthol. Soc. **21**(4): 651–659. doi:10.2307/1468436.

Moore, J.W. 2006. Animal ecosystem engineers in streams. Bioscience, **56**(3): 237–246. doi:10.1641/0006-3568(2006)056[0237:AEEIS]2.0.CO;2.

Moore, J.W., and Schindler, D.E. 2010. Spawning salmon and the phenology of emergence in stream insects. Proc. Biol. Sci. **277**(1688): 1695–1703. doi:10.1098/rspb.2009.2342. PMID:20129980.

Payne, L.X., and Moore, J.W. 2006. Mobile scavengers create hotspots of biological productivity in freshwater ecosystems. Oikos, **115**: 69–80. doi:10.1111/j.2006.0030-1299.14899.x.

Petersen, R.C., and Cummins, K.W. 1974. Leaf processing in a woodland stream. Freshw. Biol. **4**(4): 343–368. doi:10.1111/j.1365-2427.1974.tb00103.x.

Polis, G.A., Anderson, W.B., and Holt, R.D. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu. Rev. Ecol. Syst. **28**(1): 289–316. doi:10.1146/annurev.ecolsys.28.1.289.

Roberts, M.L. 2007. Sources, transport and fate of terrestrial organic matter inputs to small Puget Lowland streams: effects of urbanization, floods and salmon. Ph.D. thesis, University of Washington, Seattle, Wash., USA.

Rosemond, A.D., Swan, C.M., Kominoski, J.S., and Dye, S.E. 2010. Non-additive effects of litter mixing are suppressed in a nutrient-enriched stream. Oikos, **119**(2): 326–336. doi:10.1111/j.1600-0706.2009.17904.x.

Srivastava, D.S., Cardinale, B.J., Downing, A.L., Duffy, J.E., Jouseau, C., Sankaran, M., and Wright, J.P. 2009. Diversity has stronger top-down than bottom-up effects on decomposition. Ecology, **90**(4): 1073–1083. doi:10.1890/08-0439.1. PMID:19449701.

Swan, C.M., and Palmer, M.A. 2004. Leaf diversity alters litter breakdown in a Piedmont stream. J. N. Am. Benthol. Soc. **23**(1): 15–28. doi:10.1899/0887-3593(2004)023<0015:LDALBI>2.0.CO;2.

Sweeney, B.W., Bott, T.L., Jackson, J.K., Kaplan, L.A., Newbold, J.D., Standley, L.J., Hession, W.C., and Horwitz, R.J. 2004. Riparian deforestation, stream narrowing, and loss of stream ecosystem services. Proc. Natl. Acad. Sci. U.S.A. **101**(39): 14132–14137. doi:10.1073/pnas.0405895101. PMID:15381768.

Thorp, J.H., and Covich, A.P. 2001. Ecology and classification of North American freshwater invertebrates. 2nd ed. Academic Press, San Diego, Calif.

Tiegs, S.D., Campbell, E.Y., Levi, P.S., Ruegg, J., Benbow, M.E., Chaloner, D.T., Merritt, R.W., Tank, J.L., and Lamberti, G.A. 2009. Separating physical disturbance and nutrient enrichment caused by Pacific salmon in stream ecosystems. Freshw. Biol. **54**(9): 1864–1875. doi:10.1111/j.1365-2427.2009.02232.x.

Verspoor, J.J., Braun, D.C., Stubbs, M.M., and Reynolds, J.D. 2011. Persistent ecological effects of a salmon-derived nutrient pulse on stream invertebrate communities. Ecosphere, **2**(2): art. 18. doi:10.1890/ES10-00011.1.

Washington Department of Fish and Wildlife. 2002. South Sound – Eld Inlet fall chum stock status report. Washington Department of Fish and Wildlife, Fish Program, Olympia, Wash., USA. Available from http://wdfw.wa.gov/webmaps/salmonscape/sasi/full_stock_rpts/2195.pdf [accessed 27 April 2011].

Webster, J.R., and Benfield, E.F. 1986. Vascular plant breakdown in freshwater ecosystems. Annu. Rev. Ecol. Syst. **17**(1): 567–594. doi:10.1146/annurev.es.17.110186.003031.

Wiggins, G.B. 1996. Larvae of the North American caddisfly genera (Trichoptera). 2nd ed. University of Toronto Press, Toronto, Ont.

Willson, M.F., and Halupka, K.C. 1995. Anadromous fish as keystone species in vertebrate communities. Conserv. Biol. **9**(3): 489–497. doi:10.1046/j.1523-1739.1995.09030489.x.

Winder, M., Schindler, D.E., Moore, J.W., Johnson, S.P., and Palen, W.J. 2005. Do bears facilitate transfer of salmon resources to aquatic macroinvertebrates? Can. J. Fish. Aquat. Sci. **62**(10): 2285–2293. doi:10.1139/f05-136.

Wipfli, M.S., and Baxter, C.V. 2010. Linking ecosystems, food webs, and fish production: subsidies in salmonid watersheds. Fisheries, **35**: 373–387.

Wipfli, M.S., Hudson, J., and Caouette, J. 1998. Influence of salmon carcasses on stream productivity: response of biofilm and benthic macroinvertebrates in southeastern Alaska, U.S.A. Can. J. Fish. Aquat. Sci. **55**(6): 1503–1511. doi:10.1139/f98-031.

Yanai, S., and Kochi, K. 2005. Effects of salmon carcasses on experimental stream ecosystems in Hokkaido, Japan. Ecol. Res. **20**(4): 471–480. doi:10.1007/s11284-005-0056-7.

Zhang, Y., Negishi, J.N., Richardson, J.S., and Kolodziejczyk, R. 2003. Impacts of marine-derived nutrients on stream ecosystem functioning. Proc. R. Soc. Lond. B Biol. Sci. **270**(1529): 2117–2123. doi:10.1098/rspb.2003.2478.

CORRIGENDUM / RECTIFICATIF

Corrigendum: A new likelihood for simultaneously estimating von Bertalanffy growth parameters, gear selectivity, and natural and fishing mortality

Nathan G. Taylor, Carl J. Walters, and Steven J.D. Martell

Ref.: Can. J. Fish. Aquat. Sci. **62**(1): 215–223.

The captions for Fig. 2 and Fig. 3 are incorrect. They should read as follows:

Fig. 2. Proportional error in parameter estimates for each likelihood formulation with $F = K$. (*a*) Fabens; (*b*) model 1; (*c*) model 2; (*d*) model 3; (*e*) model 4. Circles represent outliers.

Fig. 3. Proportional error in parameter estimates for each likelihood formulation with $F = 1$. (*a*) Fabens; (*b*) model 1; (*c*) model 2; (*d*) model 3; (*e*) model 4. Circles represent outliers.

Received 7 July 2011. Accepted 7 July 2011. Published at www.nrcresearchpress.com/cjfas on 24 August 2011.
J17744

N.G. Taylor, C.J. Walters, and S.J.D. Martell. Fisheries Center, The University of British Columbia, 2259 Lower Mall, Vancouver, BC V6T 1Z4, Canada.

Corresponding author: Nathan G. Taylor (e-mail: n.taylor@fisheries.ubc.ca).

doi:10.1139/F2011-100

Published by NRC Research Press

CORRIGENDUM / RECTIFICATIF

Corrigendum: Using acoustic data from fishing vessels to estimate walleye pollock (*Theragra chalcogramma*) abundance in the eastern Bering Sea

Taina Honkalehto, Patrick H. Ressler, Richard H. Towler, and Christopher D. Wilson

Ref.: Can. J. Fish. Aquat. Sci. **68**(7): 1231–1242.

The labels of the colour scale for Fig. 4 were incorrect; they should have been logarithmic, rather than linear, as noted in the corrected version of the figure (see following page).

Received 7 July 2011. Accepted 7 July 2011. Published at www.nrcresearchpress.com/cjfas on 22 August 2011.
J21904

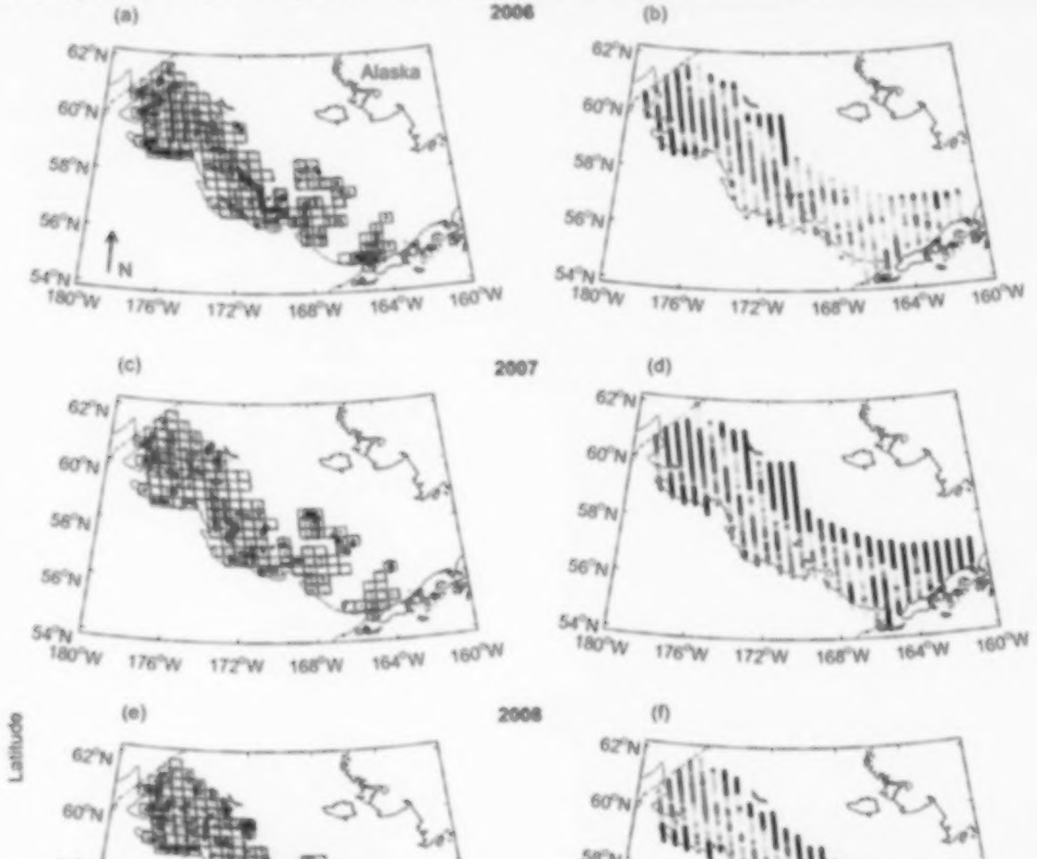
T. Honkalehto, P.H. Ressler, R.H. Towler, and C.D. Wilson. National Marine Fisheries Service, Alaska Fisheries Science Center, Resource Assessment and Conservation Engineering Division, 7600 Sand Point Way NE, Seattle, WA 98115, USA.

Corresponding author: Taina Honkalehto (e-mail: Taina.Honkalehto@noaa.gov).

doi:10.1139/F2011-101

Published by NRC Research Press

Fig. 4. Backscatter classified as walleye pollock from the bottom trawl (BT) survey index area (panels *a, c, e, g*) and the acoustic-trawl (AT) survey (panels *b, d, f, h*) in summers 2006–2009. A solid blue line indicates the 200 m depth contour. The diagonal dashed line in the upper left corner of each plot indicates the boundary between the USA and the Russian Exclusive Economic Zone.


(a)
2006
(b)
Alaska
N
(c)
2007
(d)
(e)
2008
(f)
(g)
2009
(h)
62°N
60°N
58°N
56°N
54°N
180°W
176°W
172°W
168°W
164°W
160°W
Latitude
Longitude
s_A, 38 kHz
0
10
100
1000

CORRIGENDUM / RECTIFICATIF

Corrigendum: Role of climate and invasive species in structuring trout distributions in the interior Columbia River Basin, USA

Seth J. Wenger, Daniel J. Isaak, Jason B. Dunham, Kurt D. Fausch, Charles H. Luce, Helen M. Neville, Bruce E. Rieman, Michael K. Young, David E. Nagel, Dona L. Horan, and Gwynne L. Chandler

Ref.: Can. J. Fish. Aquat. Sci. **68**(6): 988–1008.

On page 990, it was incorrectly stated that sites within basins with drainage areas larger than 400 km^2 were excluded. In fact, sites within basins with drainage areas larger than approximately 2500 km^2 were excluded. The authors apologize for any confusion to the reader.

Received 28 June 2011. Accepted 28 June 2011. Published at www.nrcresearchpress.com/cjfas on 22 August 2011.
J21957

S.J. Wenger. Trout Unlimited, 322 East Front Street, Suite 401, Boise, ID 83702, USA.
D.J. Isaak, C.H. Luce, D.E. Nagel, D.L. Horan, and G.L. Chandler. US Forest Service Rocky Mountain Research Station, 322 East Front Street, Suite 401, Boise, ID 83702, USA.
J.B. Dunham. US Geological Survey Forest and Rangeland Ecosystem Science Center, 3200 SW Jefferson Way, Corvallis, OR 97331, USA.
K.D. Fausch. Colorado State University, Department of Fish, Wildlife, and Conservation Biology, Fort Collins, CO 80523-1474, USA.
H.M. Neville. Trout Unlimited, 910 Main Street, Suite 342, Boise, ID 83702, USA.
B.E. Rieman.* US Forest Service Rocky Mountain Research Station, P.O. Box 1541, Seeley Lake, MT 59868, USA.
M.K. Young. US Forest Service Rocky Mountain Research Station, 800 East Beckwith Avenue, Missoula, MT 59801, USA.

Corresponding author: Seth J. Wenger (e-mail:swenger@tu.org).

*Retired.

doi:10.1139/F2011-102

Contents (concluded) / Table des matières (suite et fin)

Amanda J. Finger, Eric C. Anderson, Molly R. Stephens, and Bernard P. May 1369–1386 Application of a method for estimating effective population size and admixture using diagnostic single nucleotide polymorphisms (SNPs): implications for conservation of threatened Paiute cutthroat trout (*Oncorhynchus clarkii seleniris*) in Silver King Creek, California

Richard Hillary 1387–1407 Bayesian integrated survey-based assessments: an example applied to North Sea herring (*Clupea harengus*) survey data

Joshua Kurek, Russ C. Weeber, and John P. Smol 1408–1419 Environment trumps predation and spatial factors in structuring cladoceran communities from Boreal Shield lakes

Anders Løland, Magne Aldrin, Gunnhildur Högnadóttir Steinbakk, Ragnar Bang Huseby, and Jon Arne Grøttum 1420–1434 Prediction of biomass in Norwegian fish farms

Osvaldo J. Sepulveda-Villet and Carol A. Stepien 1435–1453 Fine-scale population genetic structure of the yellow perch *Perca flavescens* in Lake Erie

Jean Le Fur, Athanase Guilavogui, and Antoine Teitelbaum 1454–1469 Contribution of local fishermen to improving knowledge of the marine ecosystem and resources in the Republic of Guinea, West Africa

Helge Skoglund, Sigurd Einum, Torbjørn Forseth, and Bjørn Torgeir Barlaup 1470–1479 Phenotypic plasticity in physiological status at emergence from nests as a response to temperature in Atlantic salmon (*Salmo salar*)

William O. Hobbs, Rolf D. Vinebrooke, and Alexander P. Wolfe 1480–1494 Biogeochemical responses of two alpine lakes to climate change and atmospheric deposition, Jasper and Banff National parks, Canadian Rocky Mountains

Welles D. Bretherton, John S. Kominoski, Dylan G. Fischer, and Carri J. LeRoy 1495–1506 Salmon carcasses alter leaf litter species diversity effects on in-stream decomposition

CORRIGENDA RECTIFICATIFS

Nathan G. Taylor, Carl J. Walters, and Steven J.D. Martell 1507 Corrigendum: A new likelihood for simultaneously estimating von Bertalanffy growth parameters, gear selectivity, and natural and fishing mortality

Taina Honkalehto, Patrick H. Ressler, Richard H. Towler, and Christopher D. Wilson 1508–1509 Corrigendum: Using acoustic data from fishing vessels to estimate walleye pollock (*Theragra chalcogramma*) abundance in the eastern Bering Sea

Seth J. Wenger, Daniel J. Isaak, Jason B. Dunham, Kurt D. Fausch, Charles H. Luce, Helen M. Neville, Bruce E. Rieman, Michael K. Young, David E. Nagel, Dona L. Horan, and Gwynne L. Chandler 1510 Corrigendum: Role of climate and invasive species in structuring trout distributions in the interior Columbia River Basin, USA

Postage paid at Ottawa
Publications mail
Registration No. 40110225

Port payé à Ottawa
Poste-publication
Enregistrement n° 40110225

USPS periodical postage paid at Plattsburgh, NY 12901, USA

Canadian Journal of Fisheries and Aquatic Sciences

Volume 68, Number 8, August 2011

Journal canadien des sciences halieutiques et aquatiques

Volume 68, numéro 8, août 2011

	ARTICLES	ARTICLES
Njal Rollinson and Jeffrey A. Hutchings	1307–1315	Why does egg size of salmonids increase with the mean size of population spawning gravels?
Peter S. Levi, Jennifer L. Tank, Scott D. Tiegs, Janine Rüegg, Dominic T. Chaloner, and Gary A. Lamberti	1316–1329	Does timber harvest influence the dynamics of marine-derived nutrients in Southeast Alaska streams?
Kelton W. McMahon, Marilyn L. Fogel, Beverly J. Johnson, Leah A. Houghton, and Simon R. Thorrold	1330–1340	A new method to reconstruct fish diet and movement patterns from δ^{13}C values in otolith amino acids
Sean A. Hayes, Morgan H. Bond, Chad V. Hanson, Andrew W. Jones, Arnold J. Ammann, Jeffrey A. Harding, Alison L. Collins, Jeffrey Perez, and R. Bruce MacFarlane	1341–1350	Down, up, down and "smolting" twice? Seasonal movement patterns by juvenile steelhead (*Oncorhynchus mykiss*) in a coastal watershed with a bar closing estuary
Gretta T. Pecl, Sean R. Tracey, Leonid Danyushevsky, Simon Wotherspoon, and Natalie A. Moltschaniwskyj	1351–1360	Elemental fingerprints of southern calamary (*Sepioteuthis australis*) reveal local recruitment sources and allow assessment of the importance of closed areas
M. Joanne Morgan, Alfonso Perez-Rodriguez, and Fran Saborido-Rey	1361–1368	Does increased information about reproductive potential result in better prediction of recruitment?

Continued on inside back cover / Suite au verso

Front cover: Ihoko Saito/Toshiyuki Tajima/Dex Image/Getty Images.

Page couverture : Ihoko Saito/Toshiyuki Tajima/Dex Image/Getty Images.

NRC Research Press

CANADIAN SCIENCE PUBLISHING | ÉDITIONS SCIENCES CANADA

Printed in Canada by | Imprimé au Canada par
NRC Reprographic Services | Service de reprographie CNRC

Page production by | Mise en page par
NRC Research Press | NRC Research Press

0706-652X(201108)68:8;1-X